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Chapter 5.2

A MUTANT CATALOGUE OF *LOTUS JAPONICUS*

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Below are tables describing symbiotic, morphological, metabolic, and photorespiratory mutant lines (alleles). A more exhaustive catalogue of published and unpublished mutants and their gene mapping positions is now being prepared by Stougaard and Sandal and will soon be published elsewhere.

SYMBIOTIC MUTANTS

Mutant line	Phenotype	Description	References
LjNfr1= LjSym1-1 Ljsym1-2	Nod-, Ami+	No first responses after application of purified Nod factors (No depolarization nor extra-cellular alkalinisation). *Nfr1* encodes a receptor kinase involved in the recognition of Nod factors. The protein has a LysM domain in the extracellular moiety, involved in the recognition of the backbone of Nod-factor, a transmembrane domain, and a Ser/Thr kinase domain in the intracellular part. The simplest model is that NFR1 act together with NFR5 in the recognition of the Nod-factor.	Radutoiu et al. (2003), Nature 425, 585-592. Madsen et al. (2003), Nature 425, 637-640.
LjNfr5= LjSym5	Nod-, Ami+	No first responses after application of purified Nod factors (no depolarization or extracellular alkalinization). *Nfr5* encodes a receptor kinases involved in Nod factor recognition. The *Lj*NFR5 PK domain is atypical since it lacks the activation domain.	Radutoiu et al. (2003), Nature 425, 585-592. Madsen et al. (2003), Nature 425, 637-640.

282-287 (LjSym2-1), 282-288 (LjSym 2-2) Allelic to cac41.5 (allelic to LjEMS61 and 282-287) LjEMS61	Coi-, Nod-	Blocked infection by *Glomus intraradices* within rhizodermal cells, hyphae swelling, and deformation. Exaggerated swelling and deformation of root hairs after *Rhizobium* infection, not root hair curling, no infection threads formation. Gene cloned: SYMRK, encodes a symbiosis receptor kinase with three domains: a signal peptide, an extracellular domain comprising leucine-rich repeats, a transmembrane and an intracellular protein kinase domain. *Lotus SYMRK* is required for both rizobial and mycorrhizal symbiosis.	Schauser et al., (1998), Mol. Gen. Genet. 259, 414-423. Wegel et al. (1998), MPMI 11 , 933-936. Stracke et al. (2002), Nature 417, 959-962.
Ljsym21-1= LJEMS34, Ljsym21-2= LJEMS61 (allelic to Ljsym2)	Nod-	Fungal infections arrested in epidermal cells, hyphae swelling, and deformation. Exaggerated swelling and deformation of root hairs after *Rhizobium* infection, not root hair curling, no infection threads formation. Gene cloned: *SYMRK*, symbiosis receptor kinase.	Szczyglowski et al. (1998), MPMI 11, 684-697 Stracke et al. (2002), Nature 417, 959-962
Sym 16 = har = 1-3, (allelic to LjEMS102 = har 1-1, and Ljsym78)	Nod+++, fix+, Nts	Hyper-nodulating, nodulation resistant to nitrate, fails in autoregulation of nodulation, short and much branched root system. Gene cloned: The *har1* gene is involved in long-distance signalling of nodulation auto-regulation (shoot controlled auto-regulation of nodule number) and lateral root development. *Har1* encodes a receptor kinase with a LRR repeat, a transmembrane domain and an intracellular serine/threonine kinase domain with homology to Arabidopsis CLAVATA1	Schauser et al. (1998), Mol. Gen. Genet. 259, 414-423. Wopereis et al. (2000) The Plant Journal 23, 97-114. Krussell et al (2002), Nature 420, 422-426. Nishimura et al. (2002) Nature 420, 426-429.
LjEMS102 =Ljsym34-1 (har1-1)	Nod+++, brl, Nts	Hyper-nodulating, nodulation resistant to nitrate, fails in autoregulation of nodulation, short and much branched root system. Gene cloned: *HAR1*, receptor-like kinase involved in shoot autoregulation of nodule number.	Szczyglowski et al. (1998) MPMI 11, 684 -697. Wopereis et al. (2000) The Plant Journal 23, 97-114. Krussell et al. (2002), Nature 420, 422-426. Nishimura et al. (2002) Nature 420, 426-429. Jiang and Gresshoff, (2002) Functional

			Plant Biology 29, 1371-1376.
Ljsym78 -1 Ljsym 78-2 (alellic to Ljsym16 and har1)	Nod+++, Arb++	RS: hyper-nodulating, branched root system AMS: Stimulated growth and activity of arbuscules, but decreased growth of hyphae and vesicles	Kawaguchi et al. (2002), MPMI 15, 17-26. Solaiman et al. (2000) Journal of Plant Research, 113, 443-448.
Nin	Nod-	Arrested at the stage of bacterial recognition, nodule inception, no formation of infection threads or nodule primordia, but normal root hair curling. T-DNA tagged. Gene cloned: *Nin*, plant growth regulator of nodule initiation with a DNA binding domain. NIN protein has regional similarity to transcription factors, and the predicted DNA-binding/dimerisation domain identifies and typifies a consensus motif conserved in plant proteins with a function in nitrogen-controlled development	Schauser et al. (1999), Nature 402, 191-195.
Ljsym77= astray	Nod++	Double number of nodules on a wider area of root, early initiation of nodule development. Affected in light and gravity responses, agravitropic lateral roots. Gene cloned: *LjBzf* homologous to *A. thaliana hy5* encodes a basic leucine zipper (bZIP)-type zinc finger protein with different roles in shoot (photomorphogenic development) and roots (lateral root formation and number of nodules).	Nishimura et al. (2002), Plant Cell Physiol. 43, 853-859. Nishimura et al. (2002) PNAS 99, 15206-15210.
Ljcbp1 / T-90	Nod-	Transgenic line isolated by promoter tagging, no nodulation, affected in calcium binding protein homologue	Webb et al. (2000), MPMI 13, 606-616.
Sym8	Fix-	Small white bumps, infection threads arrested at the 2nd or 3rd cell layer into the root, ending in a balloon-like structure. T-DNA tagged.	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym13	Fix- Ami+	Affected late in nodule development. Small pink non-fixing nodules, growth of the nodules resumes after 6 weeks. T-DNA tagged.	Schauser et al. (1998), Mol. Gen. Genet. 259, 414-423.
Ljsym 3-1 LjSym 3-2	Coi-, Nod-	Blocked infection by *Glomus intraradices* within rhizodermal cells, hyphae deformation. No nodule formation.	Schauser et al. (1998), Mol. Gen. Genet. 259, 414-423. Wegel et al. (1998),

			MPMI 11, 933-936
LjSym 4-1, Ljsym4-2	Coi-, Nod-	Blocked infection by *Glomus intraradices* within epidermal cells, hyphae deformation, disorganisation, and disassembly of microtubules and microfilaments during fungal penetration attempts. No root hair curling or infection thread formation.	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423. Wegel et al. (1998) MPMI 11, 933-936. Bonfante et al. (2000) MPMI 13, 1109-1120. Genre and Bofante, (2002). Protoplasma, 219, 43-50.
Sym 6-1, Sym 6-2	Fix-	Small white bumps	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym7	Fix-, Ami+	Small white bumps	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym9	Fix-	Small white bumps	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym10	Fix-	Small white bumps	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym 11	Fix-	Young pinkish nodules, no nitrogen fixation	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym12	Fix-	Pink non fixing nodules, sot sever symptoms of nitrogen starvation: light green leaves but no red purple stems	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym14	Fix-	Pink non-fixing nodules. After 6 weeks, the main root degenerates, and adventitious roots form at the hypocotyl.	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Sym 15	Nod↓ Fix+	Reduced number of nodules and development is slower than in wild type nodules.	Schauser et al. (1998) Mol. Gen. Genet. 259, 414-423.
Ljsym22-1= LJEMS46, (allelic to Ljsym4-1 and Ljsym4-2), LjEMS40	Nod-	No macroscopically visible nodules on normal looking roots. No calcium speaking upon inoculation with *Rhizobium.* The double mutant LjEMS40 exhibits a bushy root phenotype and it is non-nodulating.	Szczyglowski et al. (1998) MPMI 11, 684 697. Harris et al. (2003), MPMI 16, 335-341.

(digenic)			
Ljsym23-1= LJEMS70, Ljsym23-2= LjEMS167	Nod-	No macroscopically visible nodules on normal looking roots	Szczyglowski et al., (1998) MPMI 11, 684 697.
Ljsym24= LJEMS76	Nod-	No macroscopically visible nodules on normal looking roots	Szczyglowski et al. (1998) MPMI 11, 684 697.
Ljsym25= LJEMS223	Nod-	No macroscopically visible nodules on normal looking roots	Szczyglowski et al. (1998) MPMI 11, 684 697.
LjEMS237	Nod-	No macroscopically visible nodules on normal looking roots	Szczyglowski et al. (1998) MPMI 11, 684 697.
Ljsym26= LJEMS247	Nod-	No macroscopically visible nodules on normal looking roots	Szczyglowski et al. (1998) MPMI 11, 684 697.
Ljsym27= LjEMS81	Nod-*	Occasional presence of few, very small, white round nodule-like structures, leaky phenotype	Szczyglowski et al. (1998) MPMI 11, 684 697.
Ljsym28 = LjEMS236	Nod-*	Occasional presence of few, very small, white round nodule-like structures, leaky phenotype	Szczyglowski et al. (1998) MPMI 11, 684 697.
LjEMS45 = LjEMS88 = LjEMS217	Nod+, Fix-	Extensive nodulation in the upper and lower part of the root, but no nitrogenase activity	Szczyglowski et al. (1998) MPMI 11, 684 697.
LjEMS126 = Ljsym30	Nod+, Fix-	Infrequent, small, white nodule-like structures without nitrogenase activity Calcium speaking but no root hair deformation upon inoculation with *Rhizobium*	Szczyglowski et al. (1998) MPMI 11, 684 697. Harris et al. (2003) MPMI 16, 335-341.
LjEMS75= Ljsym31	Nod+, Fix +/-	White nodules and some pink wild type nodules, no nitrogenase activity	Szczyglowski et al. (1998) MPMI 11, 684 -697.
LjEMS208 = Ljsym32	Nod+, Fix +/-	Small pink nodules with low nitrogenase activity, stunted and chlorotic plants	Szczyglowski et al. (1998) MPMI 11, 684 -697.

LjEMS79	Nod+, Fix +/-	Leaky phenotype: some plants developed no nodules and some plants developed white, nodule like structures or few pink nodules	Szczyglowski et al. (1998) MPMI 11, 684 -697.
Ljsym70	Nod-, Ami+	Non-nodulating, but small bumps form of roots after prolonged incubation with *M. loti*. Advanced stage in the map-based cloning approach, HEGS/AFLP saturation mapping	Kawaguchi et al. (2002) MPMI 15, 17-26. Murakmi et al. (2002) Plant and cell Physiology, special issue 43, S124.
Ljsym71-1, Ljsym71-2	Nod-, Coi-	AMS: overproduction of deformed appressoria, arrested hyphae penetration in the exodermis	Senoo et al. (2000) Plant cell Physiol. 41, 726-732. Kawaguchi et al. (2002) MPMI 15, 17-26.
Ljsym72	Nod-, Coi-	AMS: poor development of mycelium, branched appressoria, nor hyphal penetration in the epidermis, neither formation of arbuscules or vesicles.	Senoo et al. (2000) Plant Cell Physiol. 41, 726-732. Kawaguchi et al. (2002) MPMI 15, 17-26.
Ljsym73	Nod-	Nodulation at very low frequency	Kawaguchi et al. (2002) MPMI 15, 17-26
Alb1 = *Alb 2*= Ljsym74	Hist-	Empty nodules lacking vascular system, bacteria remain in enlarged infection threads and fail to differentiate into bacteroids	Imaizumi-Anraku et al. (1997) Plant and Cell Physiology 38, 871-881. Kawaguchi et al. (2002) MPMI 15, 17-26. Imaizumi et al (2000) Mol. Gen. Genet. 264, 402-410.
sen1 = Ljsym75	Fix-	Stationary endosymbiont nodule, ineffective nodules, twice the nodule number, rhizobial cells fail to develop into bacteroids, many small vacuoles, early nodule senescence. Retarded plant growth.	Kawaguchi et al. (2002) MPMI 15, 17-26. Suganuma et al. (2003) Mol. Genet. Genomics 269, 312-320.
fen1 = Ljsym76	Fix-	Bacteria differentiate into bacteroids, but nodule size fails to increase due to cell	Imaizumi-Anraku et al. (1997) Plant and

		expansion, low nitrogenase activity	Cell Physiology 38, 871-881. Kawaguchi et al. (2002) MPMI 15, 17-26.
crinkle = Ljsym79	Hist-	Affected in infection thread growth, and in pollen tube growth. Many white small bumps and only few pink nodules, crinkly trichomes, short seed pods with some aborted embryos, swollen root hairs	Kawaguchi et al. (2002) MPMI 15, 17-26. Tansengco et al. (2003) Plant Physiol. 131, 1054-1063. Tansengco et al. (2004) Plant and cell Physiology, 45, 511-520.
Sym81	Fix-	Many small vacuoles in infected cells	Kawaguchi et al. (2002) MPMI 15, 17-26.
Sym82	Nod-, Ami-	Required for symbiosis of rhizobia and arbuscular mycorrhiza	Hayashi et al. (2004) Plant and Cell Physiology, Special Issue 45, S138.
Bel		Beading nodules	Hayashi et al., unpublished results, cited in VandenBoschs and Stacey (2003) Plant Physiol. 131, 840-865
Trinity	Nod-, Ami-	*Trinity* controls nodulation and micorrhization concomitantly	Imaizumi-Anraku et al. (2004) Plant and Cell Physiology Special Issue. 45, S137.
56M			Kumagai et al. (2004) Plant and Cell Physiology, Special Issue 45, S187.

MORPHOLOGICAL MUTANTS

Mutant line	**Phenotype**	**Description**	**References**
slp	nod↓	Slippery root, lacks root hairs	Kawaguchi et al. (2002) MPMI 15, 17-

			26
rdo1	nod↓	Affected in radial organisation, thick and short root, counter clockwise-directed leaflets	Kawaguchi et al. (2002) MPMI 15, 17-26.
Pfo	Flower mutant	Flowers are replaced by a large number of sepal-like organs, without sepals or stamens, and carpels are almost absent. Mutation causes sterility. Ac-tagged mutant, *pfo* has been isolated. Itencodes a F-box protein showing similarity to *Fimbriata* (*Antirrhinum*) and UNUSUAL FLOWER ORGANS (*Arabidopsis*) and to the protein Stamina pistilloida from *Pissum*. Pfo regulates floral organ identity.	Zhang et al. (2003) The Plant Journal, 33, 607 – 619.
sleepless		Affected in nyctinastic movement, unable to close leaflets toward the adaxial side at night. Pulvini at the leaflet base are replaced by a petiole-like structure.	Kawaguchi (2003) J. Plant Res. 116, 151-154.
Stipule-less mutants			Ishikawa et al. (2004) Plant and Cell Physiology, Special Issue 45, S163.
Stem	539 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Root	29 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Architecture	189 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Flower	440 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Fruit	775 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Leaf	1268 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.
Leaflets	1008 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.

Nodules	627 mutants	TILLING	Perry et al. (2003) Plant Physiology 131, 866-871.

METABOLIC MUTANTS

Mutant line	Phenotype	Description	References
Tan1	Accumulation of leaf proanthocyanidin.	The gene *Tan1* is highly homologous to basic-helix- loop-helix (*myc*-like) genes; Expression of *TAN*1 correlates well with proanthocyanidin content in leaves. The gene is interrupted in the mutant.	Gruber et al. 2003, meeting abstract
Viridicaulis		Anthocyanine accumulation	T Aoki, unpublished results, cited in van den Boschs and Stacey (2003) Plant Physiol. 131, 840-865.

PHOTORESPIRATORY MUTANTS

Mutant line	Phenotype	Description	References
LjPR1, LjPR2	Photorespiratory mutants	Poor growth in air. Symptoms of chlorosis and some necrotic spots in leaves. Growth is restored in high CO_2. Affected in chloroplastic glutamine synthetase GS2	Orea et al. (2002), Physiol. Plant. 115, 352-361.