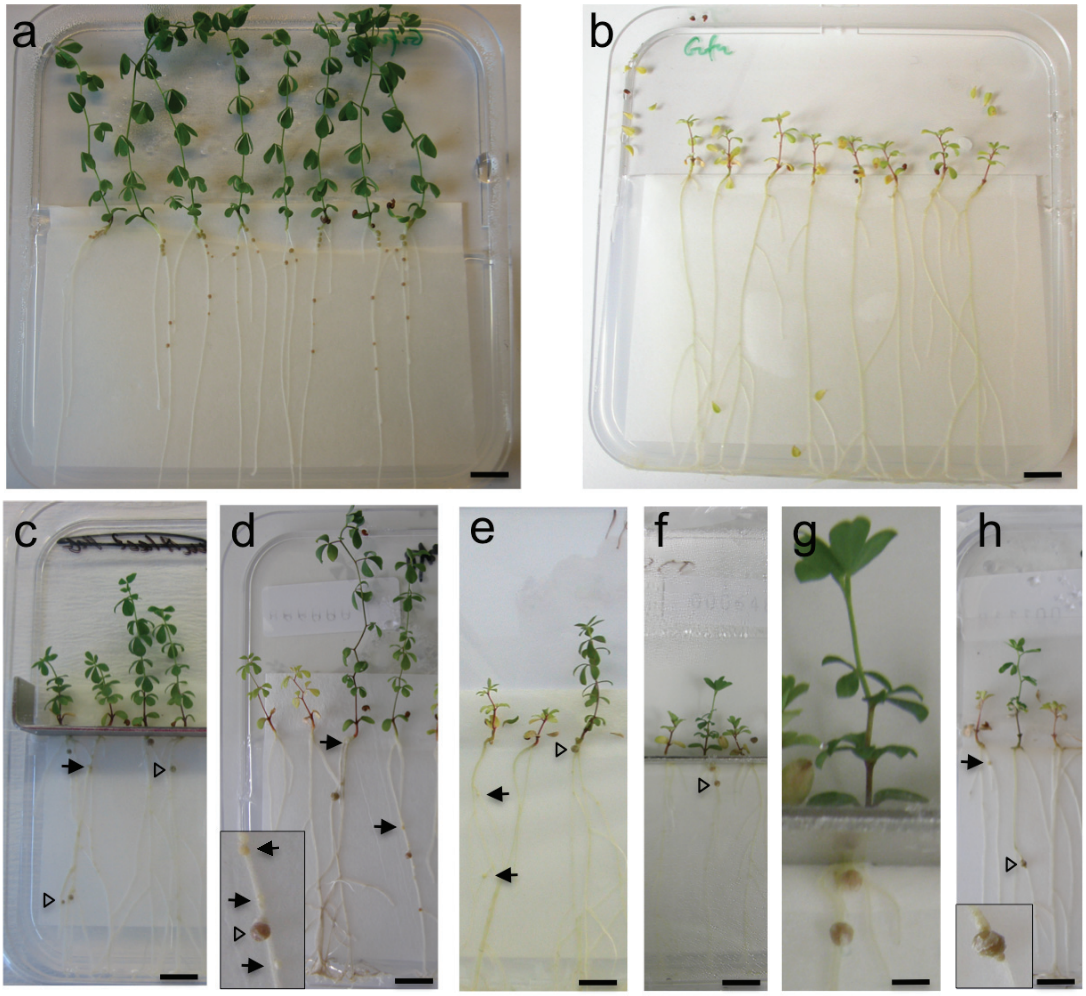
Supplementary Figure S1 Selected examples of nodulation with *M. loti* in plate experiments

Supplementary Table S1 Complete data set for *ccamk-13snf2* and *hit1snf1* experiments

Supplementary Table S2 *M. loti* strains used in this work

Supplementary Table S3 Primers for detection of *M. loti nodA* and *nodC* mutants

Supplementary Table S4 Genotyping of *Lotus* mutants



Supplementary Figure S1

Selected examples of nodulation with *M. loti* in plate experiments

(a) *L. japonicus* wild type

(b) *L. japonicus* wild type, un-inoculated

(c) *symrk-3 snf1*

(d) *nup133-3 snf1* inset: close-up of spontaneous (white) and infected red nodule

(e) *nfr5-2 snf1*

(f) *nfr1-1 nfr5-2 snf1*

(g) close-up of plant with infected nodules in (e)

(h) *nfr1-1 nfr5-2 symrk-3 snf1.* Inset: close-up of infected nodule

Arrows: examples of white, spontaneous nodules,

open triangles: examples of infected nodule

Size bars: (a- f) and(h): 1 cm, (g): 0.25 cm

Supplementary Table S1.

Plant genotype	*M. loti genotype*	white nodules	red nodules	total nodules	% red nodules	nodules/plant	red nodules/plant	# plants
ccamk-13snf2	no bacteria	86	0	86	0	0.9	0	98
ccamk-13snf2	wild type	145	0	145	0	0.6	0	249
hit1-1snf1	no bacteria	0	0	0	0	0	0	103
hit1-1snf1	wild type	30	153	183	83.6	1.4	1.2	131
ccamk-13	wild type	0	0	0	0	0	0	50
hit1	no bacteria	0	0	0	0	0	0	29
hit1	wild type	8	40	48	83.3	1.1	0.9	43
snf1	no bacteria	34	0	34	0	3.4	0	10
snf1	wild type	110	293	403	72.7	2.9	2.1	138
snf2	no bact	46	0	46	0.0	0.8	0.0	60
snf2	wild type	357	64	421	15.2	3.5	0.5	120
L. japonicus Gifu	no bacteria	0	0	0	0	0	0	40
L. japonicus Gifu	wild type	89	304	393	77.4	4.2	3.3	93

Complete data set for *ccamk-13snf2* and *hit1snf1* experiments

The epistatic relation between the *Lotus japonicus* calcium calmodulin dependent kinase gene, *CCaMK*, and the cytokinin receptor gene, *Lhk1*, was investigated in double mutants. Gain of function *snf1* (*CCaMK*) and *snf2* (*Lhk1*) alleles were combined with the loss of function *ccamk-13* and *hit1* (*Lhk1*) alleles, respectively and nodulation was scored in the absence or presence of M. *loti* bacteria. Formation of spontaneous nodules in uninoculated *ccamk-13snf2* double mutants and lack of spontaneous nodule development in uninoculated *hit1-1snf1* double mutants show that cytokinin signaling via the LHK1 receptor is operating downstream of the calcium calmodulin dependent kinase. As previously observed (Murray et al. 2007) the *hit1-1* mutants has reduced nodulation after inoculation and here we report a comparable reduction of nodulation in *hit1-1snf1* double mutants confirming the above interpretation of the epistatic relation between *CCaMK* and *Lhk1*. Under the conditions and time schedule used here inoculation enhances nodule organogenesis (mainly as white nodules) in the *snf2* mutants. A comparable enhancement was not observed in the inoculated *ccamk-13snf2* double mutants. Taken together this suggests that the effect of a relatively weak *snf2* gain of function allele is enhanced by activation of an upstream CCaMK protein following inoculation.

Supplementary Table S2. *M. loti* strains used in this work

M. loti strain	Promoter/Visible marker		Reference
R7A			Sullivan et al. 1995
R7A lacZ	*IntS*/lacZ		Sullivan et al. 1995 and this work
R7A gfp	*Tn5-nptII*/eGFP		Sullivan et al. 1995 and this work
R7A nodA			Rodpothong et al. 2009
R7A nodA	*IntS*/lacZ		Rodpothong et al. 2009
R7A nodA	*Tn5-nptII*/eGFP		Rodpothong et al. 2009
R7A nodC			Rodpothong et al. 2009
R7A nodC	*IntS*/lacZ		Rodpothong et al. 2009
R7A nodC	*Tn5-nptII*/eGFP		Rodpothong et al. 2009
NZP2235			Jarvis et al 1982
NZP2235	*hem-A*/lacZ		Schauser et al. 1999
NZP2235	*lac*/eGFP		Stuurmann et al. 2000

Supplementary Table S3 Primers for detection of *M. loti nodA* and *nodC* mutants

Bacterial locus	Forward primer	Reverse primer	Product size
nodA	5'AAGCGAGCGGATACCAGATC	5'AGGTTGTGTCACCGACAGCT	Mutant: 523 bp Wild type: 861 bp
nodC mutant	5'CGTTCAGGACGCTACTT	5' TACTGTTGCCATCTGCTCT	~1088 bp
NodC wild type	5'TACTGTTGCCATCTGCTCTT	5'GGTCAAGATTGTCGAATTGC	1678 bp

R7A nodA carries an in-frame deletion, R7A nodC is a Tn55 insertion.

Supplementary Table S4 Genotyping of *Lotus* mutants

Genotype/detection method	Type of mutation	fw primer seq	rev primer seq	temp	length	enzyme, if any	cut length mutant	cut length wt	recommended primer for sequencing
nfr1-1 CAPS or sequencing	SNP, Q493 stop	CGCTGGTTTACCCATAAACGTGTTC	GGGCAAATGCATTTGTGCTGAG	51°C	341	MseI	228+113	341	reverse
nfr5-2	LORE-2 insertion								
wild type fragment, absent in nfr5-2:		CCAGCTAGGTGATAGCTACG	CCAGAAGATGAATGCTGCTTT	54°C	391				
mutant fragment, absent in wild type		GTGACACAGTTGCCAGAGC	GCTCTATCTCTCCCAAGTCCTAGCC	54	235				
snf1-1 dCAPS or sequencing	SNP, T265I	CGCCCATAAGGGTTTGTGTTTGGG	AAAAAAAAAAACCCACTGATAACATGCTCGAGAAT	56°C	255	HinfI	255	220	forward
ccamk-13 sequencing	7 bp insertion, early stop	AGATTCCAATATTTTCAAACACTCTG	GACTAATGCCACCTAATAAGTGAG	52°C	~1000				forward
snf2 dCAPS	SNP, L266F	CACCAAAATTGCTTGGTTACCAGCAAGTTGACCGA	CCCTTCATGTGGCCCTTACCCAAC	60°C	255	XmnI	220	255	
hit1-1 sequencing		GGCAGCCAATATGAAGAGGGTG	TCAGCTGCTTCTGCTTTGAC	50	355				reverse
nup133-3	LORE-1 insertion								
wt fragment, absent in mutant		TTTGCTTCTGTCTAAGGATGTGG	TTCAAGATCTGAAACCTTACTGTA	54°C					
mutant fragment, absent i wild type		TTTGATTATCAGGACATGCCTTG	CACGAATCGAACTCTGAAACC	54°C					
nup85-1 sequencing	SNP, W306 to stop	CTGCTGTAGAAAATCTTGGAGAATGC	CTTACTGTTGTGAATGGCCTGATGTAC	56°C	359				forward
har1-3 size difference	18 bp deletion, 6 amino acid deletion	CCTGAAATGCCTATTCGTTGAG-	CACAGCTTCTTCTGCATGCG	60°C	wt563/mt545				
nin-2	transposon footprint 8 bp insertion, frameshift								
wt fragment, absent i mutant		ACCATGGACAATGAGTTGAGC	CAAGGAATTGTTGGTACAGCCTTC	62°C	257				
mutant fragment, absent in wt		CCACCATGGACAATGAGTTGACTAG	CAAGGAATTGTTGGTACAGCCTTC	62°C	259				
symrk-3	LORE-2 insertion								
wt fragment, absent in *symrk-2*		CGGCAATAGAGCGCTGGAGAGTTG	ACGCCCTTATGAAAATGTGG	54°C	251				
mutant fragment, absent in wt:		GGAGCTCTGATACCAATGTTAGG	CGGCAATAGAGCGCTGGAGAGTTG	56°C	ca. 400				
symrk-1	LORE-1 insertion								
wt fragment absent in *symrk-2*		CCATCTGTAATATGCTACTCAC	AACAGATAAATGATGCTAGGAC	52°C	557				
mutant fragment, absent in wt		AACAGATAAATGATGCTAGGAC	CACGAATCGAACTCTGAAACC	52°C					
pir-1 size difference	414 bp deletion	GATATGGATGAATGTGAAAAGTTTGACC	TTTAAGAAAGTTCTAGGTCAGGCACTC	56°C	617wt/257mt				
nap-1	LORE-1 insertion								
wt fragment, absent in *nap1-1*		CATGGCAGTCTTAGGAAGCTCTAC	TTGGTCAATACTCGACAATTCATAG	54°C					
mutant fragment, absent in wild type		CATGGCAGTCTTAGGAAGCTCTAC	CACGAATCGAACTCTGAAACC	54°C					
cerberus-3	LORE-2 insertion								
wt fragment, absent in *cerberus-3*		GCTCTTGAGATGTTCATCGTTGATC	GGACAAATCAGCAGAGTCAGGTATC	55°C					
mutant fragment, absent in wt		CAGCTTGAGGACAAGCTGAGTC	GGACAAATCAGCAGAGTCAGGTATC	55°C					
cyclops-2 sequencing	1 bp deletion, frameshift	CTCATGTTCATCTAACTTCAACAC	CTTCACCAATAGAGTCTACTTGAAC	54°C	250				reverse
castor-1 sequencing	6 bp deletion, 2 amino acid deletion	GACTGGAGTCAAAGAAGGATTACGAG	CTGTCTTGCTATAAATTAATCATGAAGCTG	54°C	287				forward
nsp1-1 sequencing	SNP, W520 to stop	GCACTGACAAACCAGCGGGAGATG	CTCATGGTGGCACATGTAACTTCTGTAC	56°C	309				forward
nsp2-3 sequencing	SNP, Q244 to stop	CCACGAACCCCAAAATGATGCTCT	CAAGTCTGGAGTGGTGGAATGAGAAGG	56°C	343				forward

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