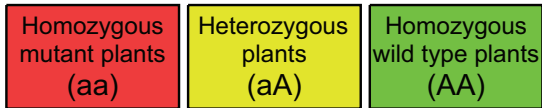
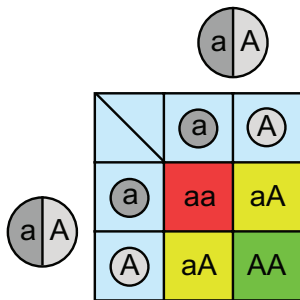


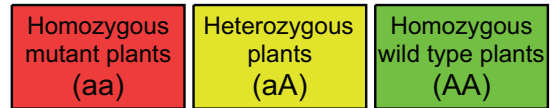
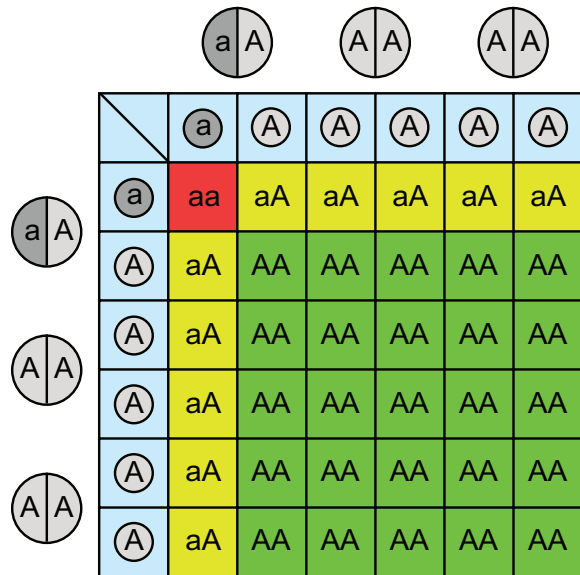
Supplemental Fig. S1. Expected segregation ratios for one vs. three cells contributing to gamete formation in an individual flower.

One germline cell contributes to gamete formation in an individual flower:



1 : 2 : 1

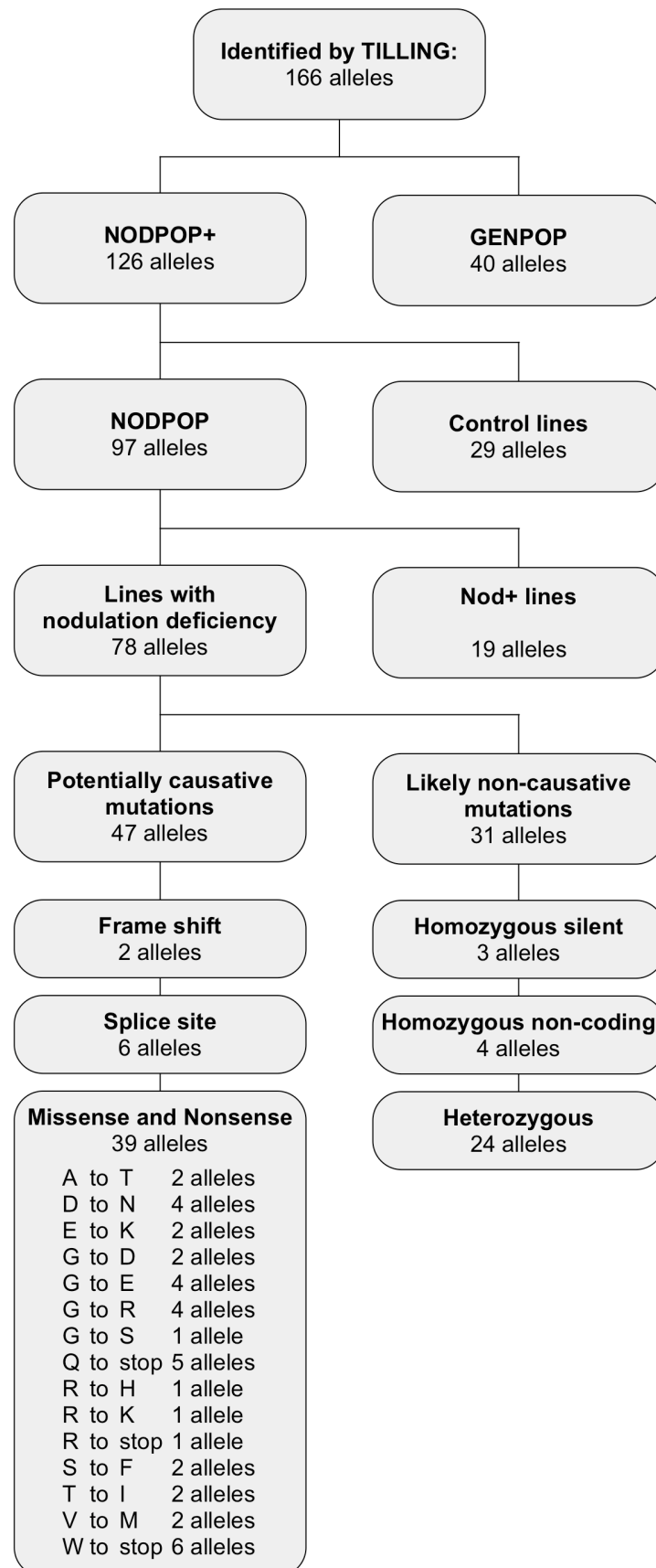
Three germline cells contribute to gamete formation in an individual flower:



1 : 10 : 25

In *Arabidopsis*, gametes in individual flowers are formed as offspring from a single germline cell (Greene et al., 2003). Therefore, a 1:2:1 segregation between homozygous mutant vs. heterozygous vs. homozygous wild type plants is observed in the M₂ offspring of a mutagenized embryo with respect to a single locus (Greene et al., 2003; left). The observed 1:10 ratio between homozygous mutant and heterozygous plants in *Lotus* could be explained by the presence of 3 germline cells in an individual flower, producing to six types of gametes in the male and female reproductive organs (right).

Supplemental Fig. S2. Overview of mutant alleles in nodulation genes identified by TILLING.



Supplemental Fig. S3:

Allelic series of symbiosis genes in *Lotus japonicus*

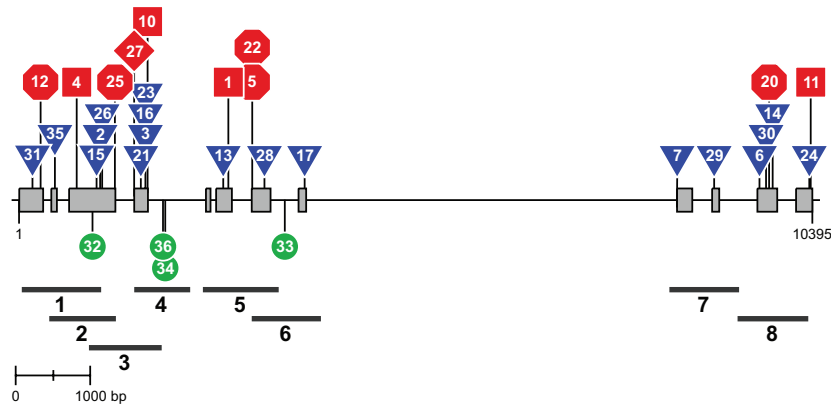
<i>CASTOR</i>	page 2
<i>CCaMK</i>	page 4
<i>CYCLOPS</i>	page 5
<i>NFR1</i>	page 6
<i>NFR5</i>	page 7
<i>NIN</i>	page 8
<i>NSP1</i>	page 9
<i>NSP2</i>	page 10
<i>NUP85</i>	page 11
<i>NUP133</i>	page 12
<i>POLLUX</i>	page 13
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References.....	page 18

Coding regions are indicated by grey boxes, non-coding regions by thin lines. Symbols above and below the genes specify the mutation position and type of the respective alleles:

red octagons	stop mutations
red diamonds	splice site mutations
red squares	deletions or insertions
blue triangles	single amino acid changes
green circles	mutations leading to synonymous exchanges or mutations in non-coding regions.

Thick black lines at the bottom indicate the regions covered by separate TILLING reactions.

Phenotype information in the tables is given for the respective progenitor/mutant line. Cases where lines harbour more than one mutation in nodulation genes are explained in footnotes.

CASTOR

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>castor-1</i>	282-227	<i>sym4-1</i>	A ₂₇₇₆ -C ₂₇₈₁ deletion	L ₄₇₉ -A ₄₈₀ deletion	-	-			Imaizumi-Anraku et al., 2005; Kistner et al., 2005
<i>castor-2</i>	EMS1749	<i>sym4-2</i>	G ₁₀₅₇ to A	A ₂₆₄ to T	-	-	NONPOP+	HOM	Imaizumi-Anraku et al., 2005; Kistner et al., 2005
<i>castor-3</i> ^d	EMS46	<i>sym22-1</i>	G ₁₆₅₅ to A	G ₃₈₃ to E	-	-	NODPOP+	HOM	Imaizumi-Anraku et al., 2005; Kistner et al., 2005
<i>castor-4</i>	25-5A	<i>sym71-1</i>	C ₇₇₈ del.	frame shift	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-5</i> ^e	24-8B; SL1937-2	<i>sym71-2</i>	G ₃₀₅₃ to A	W ₄₈₃ to stop	-;-	n.d.;			Imaizumi-Anraku et al., 2005; this work
<i>castor-6</i>	N5		C ₉₈₈₆ to T	P ₆₉₈ to L	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-7</i>	N10		G ₉₈₂₃ to A	V ₅₉₈ to I (splice site?)	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-8</i>	G00472		> 20 kb deletion	CASTOR deletion	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-9</i>	G00716		> 20 kb deletion	CASTOR deletion	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-10</i>	G00862		117 bp deletion from exon 4 to intron 4	frame shift	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-11</i>	M89-27		T ₁₀₃₇₁ del.	frame shift	-	n.d.			Imaizumi-Anraku et al., 2005
<i>castor-12</i>	SL3251-2		G ₂₇₉ to A	W ₉₃ to stop	-	-	NODPOP	HOM	Imaizumi-Anraku et al., 2005
<i>castor-13</i>	SL0820 ^f -2,3		G ₂₆₇₂ to A	D ₄₄₄ to N	(-), (-)	n.d., (-)	both in NODPOP	HET, HOM	Imaizumi-Anraku et al., 2005
<i>castor-14</i>	SL1715-2,3,4,5,6		G ₉₈₇₁ to A	A ₇₆₀ to T	-*, -, *, -, *, -*	n.d., -, n.d., n.d., n.d.	all in NODPOP	all HOM	Imaizumi-Anraku et al., 2005
<i>castor-15</i>	SL1966-3		C ₁₀₁₃ to T	T ₂₄₉ to I	-	-	NODPOP	HOM	Imaizumi-Anraku et al., 2005
<i>castor-16</i> ^d	SL3160-3		G ₁₆₅₅ to A	G ₃₈₃ to E	-	-	NODPOP	HOM	Imaizumi-Anraku et al., 2005
<i>castor-17</i>	SL6812-2		G ₃₇₃₈ to A	R ₅₉₀ to H	+/-	-	NODPOP	HOM	Imaizumi-Anraku et al., 2005
<i>castor-18</i>	KL549		n.d.	n.d.	-	n.d.			Sandal et al., 2006
<i>castor-19</i>	G00532-21		>90 kb del.	CASTOR del.	-	n.d.			Sandal et al., 2006
<i>castor-20</i>	LKL186-4		C ₉₈₂₇ to G	S ₇₄₅ to stop	-	n.d.	NODPOP+	HOM	Sandal et al., 2006
<i>castor-21</i>	B32-BA ^f		G ₁₅₉₄ to A	E ₃₆₃ to K	(-)	(+)			Murray et al., 2006
<i>castor-22</i> ^e	S41-1 ^g ; SL1937-2		G ₃₀₅₃ to A	W ₄₈₃ to stop	(-);-	(+);-			Murray et al., 2006
<i>castor-23</i> ^d	B5-2 ^g		G ₁₆₅₅ to A	G ₃₈₃ to E	(-)	(-)			Murray et al., 2006
<i>castor-24</i>	B68-B ^g ^h		G ₁₀₃₅₃ to A	W ₈₄₀ to stop	(-)	(-)			Murray et al., 2006; this work
<i>castor-25</i>	S46-1 ⁱ		G ₁₂₅₁ to A	W ₃₂₈ to stop	(-)	(-)	NODPOP+	HOM	Murray et al., 2006

<i>castor-26</i>	SL6908-2	G ₁₀₈₈ to A	G ₂₇₄ to D	-	-	NODPOP	HOM	this work
<i>castor-27</i>	SL5136-3	G ₁₅₀₃ to A	splice site	-	-	NODPOP	HOM	this work
<i>castor-28</i>	282-643M1M1M3 ^k	A ₃₂₁₄ to G	D ₅₃₇ to G	(-)	n.d.	NODPOP+	HOM	N. Sandal and J. Stougaard, pers. communication; this work
<i>castor-29</i>	SL5210-13	G ₉₁₀₆ to A	E ₆₇₂ to K	-*	n.d.	NODPOP	HET	this work
<i>castor-30</i>	SL1415-2,3,4	G ₉₇₈₅ to A	S ₇₃₁ to N	-,*,*	-,n.d.,n.d.	all in NODPOP	all HET	this work
<i>castor-31</i>	SL5768-3	C ₁₇₂ to G	R ₅₈ to G	+/-*	n.d.			this work
<i>castor-32</i>	SL6453-2	T ₉₆₀ to A	V ₂₃₁ to V	-	n.d.			this work
<i>castor-33</i>	SL1371-2	G ₃₄₈₁ to A	intron 7	-*	n.d.	NODPOP	HET	this work
<i>castor-34</i>	SL6077-2	G ₁₉₁₅ to A	intron 4	+/-*	n.d.	NODPOP	HOM	this work
<i>castor-35</i>	SL1052-1	G ₄₈₈ to A	E ₁₂₂ to K	n.d.	n.d.	GENPOP	HET	this work
<i>castor-36</i>	SL0818-1	C ₃₃₅₄ to T	intron 7	n.d.	n.d.	GENPOP	HET	this work

^a The *CASTOR* genomic sequence is deposited to GenBank under accession number AB162016 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d *castor-3*, *castor-16* and *castor-23* are identical alleles.

^e *castor-5* and *castor-22* are identical alleles.

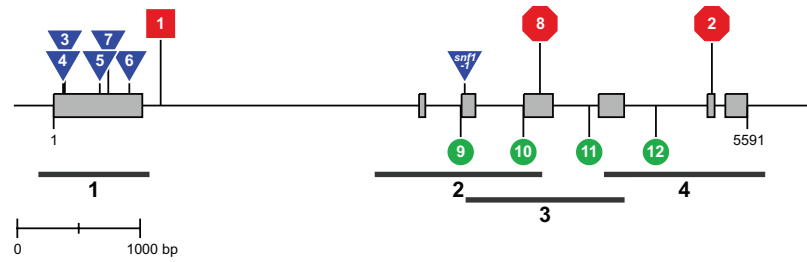
^f Lines SL0820-2,3 carry the mutant alleles *castor-13* and *ccamk-8*.

^g Lines contain in addition a *har1-1* mutation.

^h Line B68-B carries an additional heterozygous mutation A₃₀₂₉ to G in intron 6.

ⁱ Line S46-1 carries the mutant alleles *castor-25*, *nin-10*, and *har1-1*.

^k Line 282-643M1M1M3 carries the mutant alleles *castor-28*, *ccamk-7*, and *nup85-9*.

CCaMK

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>ccamk-1</i>	282-1078	<i>sym15-1</i>	tandem repeat insertion of frag- ment G564-T862	intron 1	+/-	n.d.			Schauser et al., 1998; Demchenko et al., 2004; Tirichine et al., 2006a
<i>ccamk-2</i>	cac57.3	<i>sym15-2</i>	G5307 to T	E453 to stop	-	n.d.			Demchenko et al., 2004; Kistner et al., 2005; Tirichine et al., 2006a
<i>ccamk-3</i>	8-1G	<i>sym72</i>	G89 to A	G30 to E	-	n.d.			Senoo et al., 2000; Kawaguchi et al., 2002; Tirichine et al., 2006a
<i>ccamk-4</i>	N20		C75 to T	S25 to F	n.d.	n.d.			Tirichine et al., 2006a
<i>ccamk-5</i>	B91-B ^d		G371 to A	G124 to D	(-)	(-)	NODPOP+	HOM	Murray et al., 2006
<i>ccamk-6</i>	S95-B ^d		G610 to A	G204 to R	(-)	(-)	NODPOP+	HOM	Murray et al., 2006
<i>ccamk-7</i>	282- 643M1M1M3 ^e		C439 to T	R147 to C	(-)	n.d.	NODPOP+	HET	N. Sandal and J. Stougaard, pers. communication; this work
<i>ccamk-8</i>	SL0820 ^f -2,3,4		G3920 to A	W336 to stop	(*),(-),-*	n.d.,(-),n.d.	all in NODPOP	all HOM	this work
<i>ccamk-9</i>	SL6438-2,3,4		C3282 to T	intron 2	n.d.,-*,n.d.	n.d.,n.d.,n.d.	all in NODPOP	all HET	this work
<i>ccamk-10</i>	SL0240-2		G3786 to A	intron 3	+/-	n.d.	NODPOP	HOM	this work
<i>ccamk-11</i>	SL5820-2		C4319 to T	intron 4	+	n.d.	NODPOP	HET	this work
<i>ccamk-12</i>	SL5536-2		C4756 to T	intron 5	+/-*	n.d.	NODPOP	HET	this work
<i>ccamk-13</i>	cac57.9		7 bp insertion after G462	frame shift	-	n.d.			L.H. Madsen and J. Stougaard, pers. communication
<i>snf1-1</i>	SL0467		C3314 to T	T265 to I	snf ^g	n.d.			Tirichine et al., 2006a; 2000b

^a The *CCaMK* genomic sequence is deposited to GenBank under accession number AM230792 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

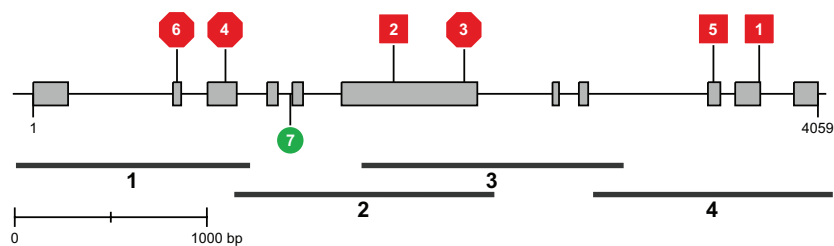
^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Lines contain in addition a *har1-1* mutation.

^e Line 282-643M1M1M3 carries the mutant alleles *castor-28*, *ccamk-7*, and *nup85-9*.

^f Lines SL0820-2,3 carry the mutant alleles *castor-13* and *ccamk-8*.

^g Spontaneous nodule formation.

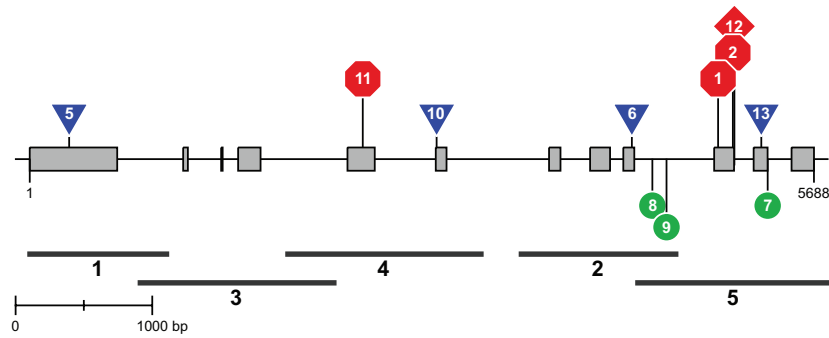
CYCLOPS

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>cyclops-1</i>	10512.9	<i>sym6-1</i>	insertion at the end of exon 10	frame shift	-	-			Schauser et al., 1998; Kistner et al., 2005; Yano et al., 2008
<i>cyclops-2</i>	1962-124; pASac124	<i>sym6-2</i>	A ₁₈₆₆ del.	frame shift	-;n.d.	-;n.d.	pASac124 in NODPOP+	HOM	Schauser et al., 1998; Yano et al., 2008; Sandal et al., 2006; this work
<i>cyclops-3</i>	EMS126	<i>sym30</i> , <i>sym6-3</i>	G ₂₂₄₁ to A	W ₃₇₁ to stop	-	-	NODPOP+	HOM	Schauser et al., 1998; Szczygłowski et al., 1998; Kistner et al., 2005; Yano et al., 2008
<i>cyclops-4</i>	N-4	<i>sym82</i>	C ₉₉₆ to T	Q ₁₀₇ to stop	-	-			Yano et al., 2006; 2008
<i>cyclops-5</i>	SL1347-2		G ₃₅₀₈ del.	frame shift	+/-	n.d.	NODPOP	HOM	Yano et al., 2008; this work
<i>cyclops-6</i>	SL0488-3		C ₇₅₂ to T	R ₇₄ to stop	*	n.d.	NODPOP	HOM	this work
<i>cyclops-7</i>	SL0295-2		C ₁₃₂₈ to T	intron 4	+/-	+	NODPOP	HET	this work

^a The *CYCLOPS* genomic sequence is deposited to GenBank under accession number AP009158 and is used as *Lotus japonicus* MG20 reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

NFR1

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nfr1-1</i>	282-118	<i>sym1-1</i>	C ₄₉₇₃ to T	Q ₄₉₃ to stop	-	+			Schauser et al., 1998; Radutoiu et al., 2003; Sandal et al., 2006
<i>nfr1-2</i>	282-665	<i>sym1-2</i>	G ₅₀₈₁ to T	E ₅₂₉ to stop	-	+			Schauser et al., 1998; Radutoiu et al., 2003; Sandal et al., 2006
<i>nfr1-3</i>	N38		n.d.	n.d.	n.d.	n.d.			Sandal et al., 2006
<i>nfr1-4</i>	G71-23		n.d.	n.d.	n.d.	n.d.			Sandal et al., 2006
<i>nfr1-5</i>	SL0456-2 ^d		G ₂₈₄ to A	G ₉₆ to D	(+)	n.d.	NODPOP	HET	this work
<i>nfr1-6</i>	SL5426-3 ^e		G ₄₃₇₇ to A	G ₄₈₅ to E	(-)	(+)	NODPOP	HOM	this work
<i>nfr1-7</i>	SL0355-3 ^f		G ₅₃₃₁ to A	K ₅₆₉ to K (splice site?)	(-)	(+)	NODPOP	HOM	this work
<i>nfr1-8</i>	SL1000-2		G ₄₆₂₃ to A	intron 9	-*	n.d.	NODPOP	HOM	this work
<i>nfr1-9</i>	SL6832-2		G ₄₆₁₉ to A	intron 9	+/-*	n.d.	NODPOP	HET	this work
<i>nfr1-10</i>	SL1174-2		G ₂₉₄₁ to A	A ₃₅₀ to T	-	+	NODPOP	HOM	this work
<i>nfr1-11</i>	SL1977-4		C ₂₄₁₂ to T	Q ₃₁₆ to stop	-	n.d.	NODPOP	HOM	this work
<i>nfr1-12</i>	SL6793-2		G ₅₁₀₅ to A	splice site	-	n.d.		HOM	this work
<i>nfr1-13</i>	SL1503-3,4,5,6		G ₅₂₈₇ to A	D ₅₅₅ to N	-*, -, -, -	n.d., -, +, -	all in NODPOP	all HET	this work

^a The *NFR1* genomic sequence is deposited to GenBank under accession number AJ575247 (*NFR1b*) and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

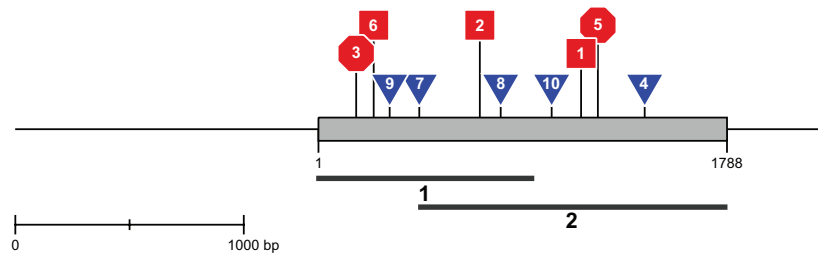
^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Line SL0456-2 carries the mutant alleles *nfr1-5* and *pollux-28*.

^e Line SL5426 carries the mutant alleles *nfr1-6*, *nfr5-6* and *nin-9*.

^f Line SL0355-3 carries the mutant alleles *nfr1-7* and *nup133-18*.

NFR5

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nfr5-1</i>	282-894		G ₁₁₃₈ -T ₁₁₆₄ deletion	E ₃₈₀ -A ₃₈₈ deletion	-	+			Madsen et al., 2003; Sandal et al., 2006
<i>nfr5-2</i>	KL865	<i>sym5</i>	transposon insertion at C ₇₀₀	frame shift	-	+			Madsen et al., 2003; Sandal et al., 2006
<i>nfr5-3</i>	EMS223; SL3257-2	<i>sym25</i>	C ₁₆₃ to T	Q ₅₅ to stop	-;-	+;+	SL3257-2 in NODPOP	SL3257-2 is HOM	Madsen et al., 2003; Sandal et al., 2006; this work
<i>nfr5-4</i>	S4-1 ^d		G ₁₄₁₅ to A	G ₄₇₂ to E	(-)	(+)			Madsen et al., 2003; Murray et al., 2006
<i>nfr5-5</i>	S58-1 ^d		G ₁₂₁₂ to A	W ₄₀₄ to stop	(-)	(+)			Madsen et al., 2003; Murray et al., 2006
<i>nfr5-6</i>	B31-E ^d ; SL5426 ^e		C ₂₃₇ del.	frame shift	(-);(-)	(+);n.d.	NODPOP+; NODPOP	HOM; HOM	Madsen et al., 2003; Murray et al., 2006; this work
<i>nfr5-7</i>	SL1093-2		G ₄₃₆ to A	V ₁₄₆ to I	+*	n.d.	NODPOP	HOM	this work
<i>nfr5-8</i>	SL1719-2 ^f ,4,5		G ₇₉₀ to A	G ₂₆₄ to R	(+/-), +/-, +/-	(+), +, n.d.	all in NODPOP	HOM, HET, HET	this work
<i>nfr5-9</i>	SL5390-2		G ₃₀₂ to A	C ₁₀₁ to Y	-	+			this work
<i>nfr5-10</i>	SL1999-4		G ₁₀₀₉ to A	A ₃₃₇ to T	n.d.	n.d.	NODPOP	HET	this work

^a The *NFR5* genomic sequence is deposited to GenBank under accession number AJ575254 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

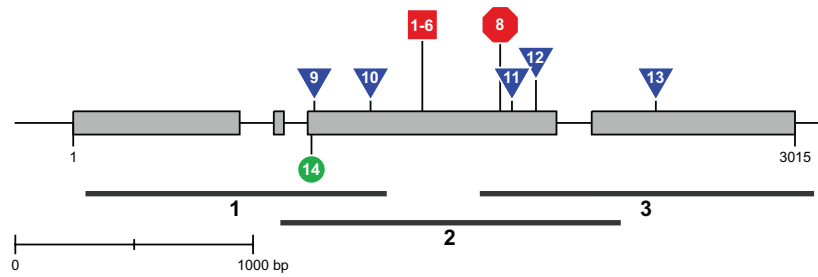
^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Lines contain in addition a *har1-1* mutation.

^e Line SL5426 carries the mutant alleles *nfr1-6*, *nfr5-6* and *nin-9*.

^f Line SL1719-2 carries the mutant alleles *nfr5-8* and *nsp1-5*.

NIN

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nin-1</i>	96.1M2		transposon insertion at C ₁₄₅₉	frame shift	-	+			Schauser et al., 1999; Sandal et al., 2006
<i>nin-2</i>	96.1M2 offspring 1		transposon footprint at C ₁₄₅₉	frame shift	-	+			Schauser et al., 1999
<i>nin-3</i>	96.1M2 offspring 2		transposon footprint at C ₁₄₅₉	frame shift	-	+			Schauser et al., 1999
<i>nin-4</i>	96.1M2 offspring 3		transposon footprint at C ₁₄₅₉	amino acid insertion V ₄₁₀ -N ₄₁₁	+	+			Schauser et al., 1999
<i>nin-5</i>	96.1M2 offspring 4		transposon footprint at C ₁₄₅₉	amino acid insertion N ₄₁₀	+	+			Schauser et al., 1999
<i>nin-6</i>	KL773		transposon footprint at C ₁₄₅₉	amino acid insertion V ₄₁₀ -N ₄₁₁	+	n.d.			N. Sandal, pers. communication
<i>nin-7</i>	KL577		n.d.	n.d.	-	+			Sandal et al., 2006
<i>nin-8</i>	B21-1 ^e ; B47-B		C ₁₇₈₅ to T	Q ₂₁₉ to stop	(-);n.d.	(-);n.d.	both in NODPOP+	both HOM	Murray et al., 2006; this work
<i>nin-9</i>	SL5369 ^e ; SL5426 ^f		G ₁₀₀₂ to A	V ₂₅₈ to M	(+);(-)	n.d.;n.d.	both in NODPOP	HOM, HOM	this work
<i>nin-10</i>	S46-1 ^g		G ₁₂₄₂ to A	A ₃₃₈ to T	(-)	(-)	NODPOP+	HOM	Murray et al., 2006; this work
<i>nin-11</i>	SL0605-2,3 ^h		G ₁₈₄₈ to A	E ₅₄₀ to K	(-),(-*)	(-),n.d.	NODPOP	HOM	this work
<i>nin-12</i>	SL1798-2,4,5		G ₁₉₂₇ to A	R ₃₆₆ to K	+,+,+*	n.d.,n.d.,n.d.	all in NODPOP	HET, HOM, HET	this work
<i>nin-13</i>	SL3012-1		G ₂₄₃₁ to A	G ₆₈₅ to R	n.d.	n.d.	GENPOP	HOM	this work
<i>nin-14</i>	SL5800-3		G ₉₈₆ to A	E ₂₅₂ to E	-*	n.d.	NODPOP	HET	this work

^a The *NIN* genomic sequence is deposited to GenBank under accession number AJ238956 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

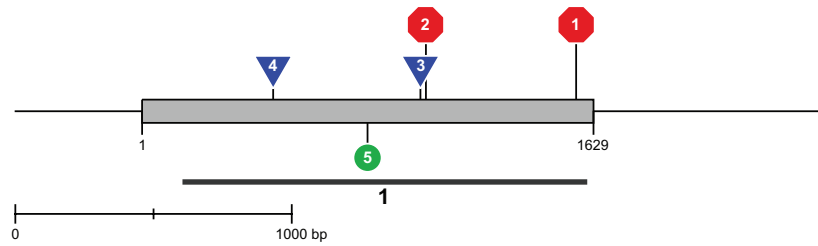
^d Line contains in addition a *har1-1* mutation.

^e Line SL5369 carries the mutant alleles *nin-9* and *nsp2-9*.

^f Line SL5426 carries the mutant alleles *nfr1-6*, *nfr5-6* and *nin-9*.

^g Line S46-1 carries the mutant alleles *castor-25*, *nin-10*, and *har1-1*.

^h Lines SL0605-2,3 carry the mutant alleles *nin-11* and *symrk-9*

NSP1

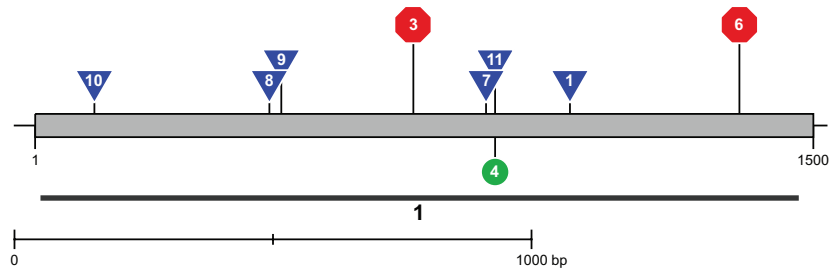
Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nsp1-1</i>	SL1795-4		G ₁₅₆₀ to A	W ₅₂₀ to stop	-	+			Heckmann et al., 2006
<i>nsp1-2</i>	S70-1		C ₁₀₂₄ to T	Q ₃₄₂ to stop	n.d.	n.d.	NODPOP+	HOM	K. Szczyglowski, pers. communication; this work
<i>nsp1-3</i>	SL0074-3		C ₁₀₀₇ to T	S ₃₃₆ to F	+*	n.d.	NODPOP	HOM	this work
<i>nsp1-4</i>	SL0241-2		A ₄₇₆ to G	N ₁₅₉ to S	+*	n.d.	NODPOP	HET	this work
<i>nsp1-5</i>	SL1719-2 ^d		G ₇₉₈ to A	S ₂₆₆ to S	(+/-)	(+)	NODPOP	HOM	this work

^a The *NSP1* genomic sequence is deposited to GenBank under accession number EF012819 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Line SL1719-2 carries the mutant alleles *nfr5-8* and *nsp1-5*.

NSP2

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nsp2-1</i>	cac18.4		T ₁₀₇₅ to A	V ₃₅₉ to E	-	+			Kawaguchi et al., 2002; Heckmann et al., 2006; Murakami et al., 2007
<i>nsp2-2</i>	[<i>sym35/sym70</i>]	<i>sym35</i> , <i>sym70</i>	large deletion	<i>NSP2</i> del.	-	+			Kawaguchi et al., 2002; Heckmann et al., 2006; Murakami et al., 2007
<i>nsp2-3</i>	SL0781-3		C ₇₃₀ to T	Q ₂₄₄ to stop	-	+	NODPOP	HOM	Heckmann et al., 2006
<i>nsp2-4</i>	SL2663-3		C ₈₈₈ to T	P ₂₉₆ to P	-*	n.d.	NODPOP	HET	this work
<i>nsp2-5</i>	B32-A ^d		n.d.	n.d.	(-)	(+)			Murray et al., 2006
<i>nsp2-6</i>	B85-E ^d		C ₁₃₅₇ to T	Q ₄₅₃ to stop	(-)	(+)			Murray et al., 2006
<i>nsp2-7</i>	B90-A ^d		C ₈₆₉ to T	A ₂₉₀ to V	(-)	(+)			Murray et al., 2006
<i>nsp2-8</i>	SL1898-4		G ₄₅₅ to A	G ₁₅₂ to D	++	n.d.	NODPOP	HET	this work
<i>nsp2-9</i>	SL5369 ^e		C ₄₇₉ to T	A ₁₆₀ to V	(+)	n.d.	NODPOP	HOM	this work
<i>nsp2-10</i>	SL1055-1		C ₁₁₃ to T	S ₃₈ to F	n.d.	n.d.	GENPOP	HET	this work
<i>nsp2-11</i>	S58-1		C ₈₈₆ to T	P ₂₉₆ to S	n.d.	n.d.	NODPOP+	HOM	K. Szczyglowski, pers. communication; this work

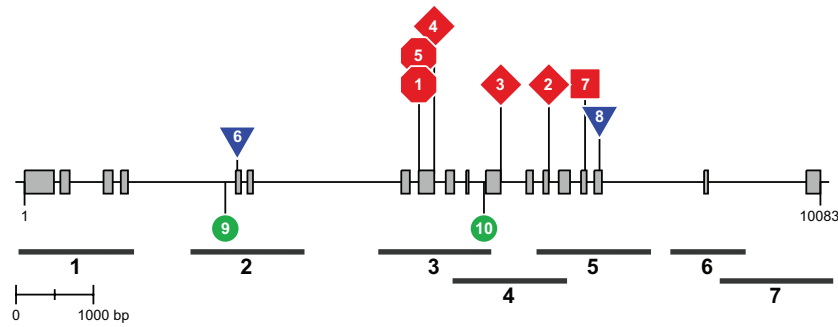
^a The *NSP2* genomic sequence is deposited to GenBank under accession number DQ665943 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star denotes additional screening in the M₃ generation. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Lines contain in addition a *har1-1* mutation.

^e Line SL5369 carries the mutant alleles *nin-9* and *nsp2-9*.

NUP85

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nup85-1^d</i>	EMS76	<i>sym24</i>	G ₄₉₉₂ to A	W ₃₀₆ to stop	-	-			Saito et al., 2007
<i>nup85-2</i>	1-1E	<i>sym73</i>	G ₆₆₂₃ to A	splice site, deletion of exon 13	-	+/-			Saito et al., 2007
<i>nup85-3</i>	1-6F	<i>sym85</i>	G ₆₀₂₀ to A	splice site, leads to stop	-	-			Saito et al., 2007
<i>nup85-4</i>	B46-D ^e		G ₅₁₈₈ to A	splice site	(-)	(-)			Murray et al., 2006; Saito et al., 2007
<i>nup85-5^d</i>	EMS76		G ₄₉₉₂ to A	W ₃₀₆ to stop	-	-	NODPOP+	HOM	Kistner et al., 2005; this work
<i>nup85-6</i>	SL0948-9		C ₂₇₀₁ to T	S ₂₃₅ to F	+/-*	n.d.	NODPOP	HOM	this work
<i>nup85-7</i>	Pgus7M1M1		A ₇₁₀₈ del.	frame shift	+/-	n.d.	NODPOP+	HOM	N. Sandal and J. Stougaard, pers. communication; this work
<i>nup85-8</i>	SL0206-3		C ₇₂₈₂ to T	S ₆₂₅ to F	+	n.d.	NODPOP	HET	this work
<i>nup85-9</i>	282-643M1M1M3 ^f		T ₂₅₄₅ to C	intron 4	(-)	n.d.	NODPOP+	HET	N. Sandal and J. Stougaard, pers. communication; this work
<i>nup85-10</i>	SL0005-2		C ₅₈₄₈ to T	intron 10	+*	n.d.	NODPOP	HOM	this work

^a The *NUP85* genomic sequence is deposited to GenBank under accession number AP009253 and is used as *Lotus japonicus* MG20 reference. Adenine of the ATG initiator codon is designated as position 1.

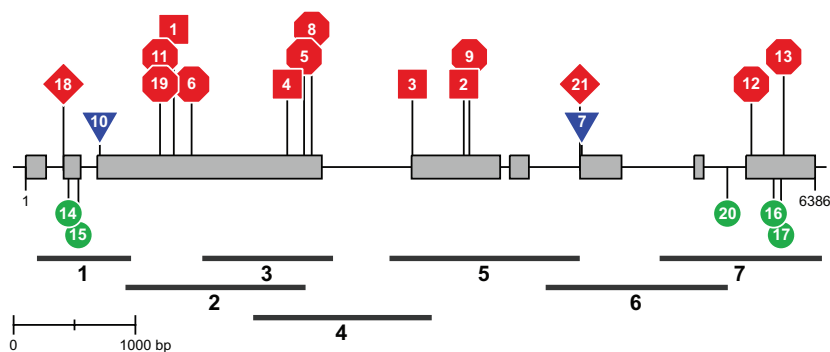
^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour, in the AM phenotype it designates delayed colonization. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d *nup85-1* and *nup85-5* are the same allele in the same line.

^e Lines contain in addition a *har1-1* mutation.

^f Line 282-643 carries the mutant alleles *castor-28*, *ccamk-7*, and *nup85-9*.

NUP133

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>nup133-1</i>	5371-22	<i>sym3-1</i>	A ₁₁₉₆ -T ₁₂₀₅ deletion	frame shift	-	-			Schauser et al., 1998; Kanamori et al., 2006
<i>nup133-2</i>	2557-1	<i>sym3-2</i>	T ₃₅₄₃ -T ₃₅₄₄ deletion	frame shift	-	-			Schauser et al., 1998; Kanamori et al., 2006
<i>nup133-3</i>	cac33.1	<i>sym3-3</i>	transposon insertion at T ₃₁₂₆	frame shift	-	-			Schauser et al., 1998; Kanamori et al., 2006
<i>nup133-4^d</i>	EMS247 ^d	<i>sym45^d</i>	T ₂₁₁₅ del.	frame shift	-	-			Schauser et al., 1998; Kanamori et al., 2006
<i>nup133-5</i>	B46-C ^e		G ₂₂₅₃ to A	W ₆₅₆ to stop	n.d.	n.d.			Murray et al., 2006
<i>nup133-6</i>	B62-D ^e		G ₁₃₄₂ to A	W ₃₅₂ to stop	(+/-)	(-)			Murray et al., 2006
<i>nup133-7</i>	B80-A ^e		G ₄₄₉₆ to A	E ₁₀₂₉ to K	(-)	(-)			Murray et al., 2006
<i>nup133-8</i>	B88-B ^{2e}		C ₂₃₂₁ to A	Q ₆₇₉ to stop	(-)	(-)			Murray et al., 2006
<i>nup133-9</i>	Sup12 ^e		G ₃₅₈₇ to A	W ₆₅₈ to stop	(+/-)	(-)			Murray et al., 2006
<i>nup133-10</i>	SL0886		G ₆₀₀ to A	G ₁₀₅ to D	+	n.d.	NODPOP	HET	this work
<i>nup133-11</i>	SL5179-12		C ₁₀₉₇ to T	Q ₂₇₁ to stop	-	n.d.	NODPOP	HOM	this work
<i>nup133-12</i>	SL0443-3		C ₅₈₇₄ to T	Q ₁₁₄₀ to stop	-	-	NODPOP	HOM	this work
<i>nup133-13</i>	B30-A		T ₆₁₅₈ to G	Y ₁₂₃₄ to stop	-	-	NODPOP+	HET	K. Szczygłowski, pers. communication; this work
<i>nup133-14</i>	SL0317-3 ^f		G ₃₄₀ to A	E ₆₇ to E	(++)	n.d.	NODPOP	HET	this work
<i>nup133-15</i>	SL0615-2		G ₄₁₈ to A	L ₉₃ to L	-	n.d.	NODPOP	HOM	this work
<i>nup133-16</i>	SL5390-2		G ₆₀₆₂ to A	K ₁₂₀₂ to K	-	+	NODPOP	HET	this work
<i>nup133-17</i>	SL1409-2		C ₆₁₀₈ to T	L ₁₂₁₈ to L	+	n.d.	NODPOP	HET	this work
<i>nup133-18</i>	SL0355-3 ^g		G ₃₀₉ to A	splice site	(-)	(+)			this work
<i>nup133-19</i>	SL2032-2; SL4119-2; SL5064-2;		G ₁₀₉₆ to A	W ₂₇₀ to stop	-; -; -	n.d.; n.d.; n.d.			this work
<i>nup133-20</i>	SL0836-2		G ₅₆₈₀ to A	intron 7	-*	n.d.	NODPOP	HET	this work
<i>nup133-21^d</i>	EMS247 ^d	<i>sym45^d</i>	G ₄₄₉₆ to A	splice site	n.d.	n.d.			Schauser et al., 1998; Kanamori et al., 2006; this work

^a The *NUP133* genomic sequence is deposited to GenBank under accession number AJ890252 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

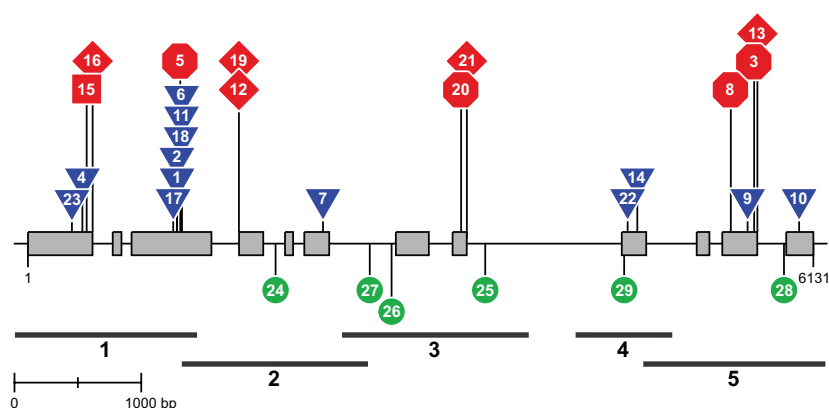
^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d Line EMS247 carries two mutations marked as mutant alleles *nup133-4* and *nup133-21*.

^e Lines contain in addition a *har1-1* mutation.

^f Line SL0317-3 carries the mutant alleles *nup133-14* and *pollux-18*.

^g Line SL0355-3 carries the mutant alleles *nfr1-7* and *nup133-18*.

POLLUX

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>pollux-1^d</i>	EMS70	<i>sym23-1</i>	G1152 to A	G303 to S	-	-	NODPOP+	HOM	Imaizumi-Anraku et al., 2005
<i>pollux-2^d</i>	EMS167	<i>sym23-2</i>	G1152 to A	G303 to S	-	-	NODPOP+	HOM	Imaizumi-Anraku et al., 2005
<i>pollux-3</i>	29-2A	<i>sym86-1</i>	T568 to A	L841 to stop	-	n.d.			Imaizumi-Anraku et al., 2005
<i>pollux-4</i>	SL0571-2		C434 to T	S145 to F	-	-	NODPOP	HET	Imaizumi-Anraku et al., 2005
<i>pollux-5</i>	SL3130-2,4		G1204 to A	W320 to stop	-*, -	n.d., -	both in NODPOP	both HOM	Imaizumi-Anraku et al., 2005
<i>pollux-6</i>	SL5691-3,4		C1210 to T	S322 to F	+, +*	n.d., n.d.	both in NODPOP	both HOM	Imaizumi-Anraku et al., 2005
<i>pollux-7</i>	SL1899-2,4		G2313 to A	G530 to E	+/-, +	n.d., n.d.	both in NODPOP	both HOM	Imaizumi-Anraku et al., 2005
<i>pollux-8</i>	SL0159-2,3,5,6		G5486 to A	W780 to stop	-*, -*, -*	n.d., n.d., -*	all in NODPOP	all HOM	Imaizumi-Anraku et al., 2005
<i>pollux-9</i>	SL0405-2,3,5,6		G5634 to A	E330 to K	-*, -*, -*	n.d., n.d., n.d., n.d.	all in NODPOP	all HOM	Imaizumi-Anraku et al., 2005
<i>pollux-10</i>	SL1070-2		G6021 to A	G882 to S	-	n.d.	NODPOP	HOM	Imaizumi-Anraku et al., 2005
<i>pollux-11</i>	B12-1A ^e		G1205 to A	W320 to stop	(-)	(-)			Murray et al., 2006
<i>pollux-12^f</i>	B50-C ^e		G1644 to A	splice site	(+/-)	(-)	NODPOP+	HOM	Murray et al., 2006
<i>pollux-13</i>	S49-D ^e		G5682 to A	splice site	(-)	(-)	NODPOP+	HOM	Murray et al., 2006
<i>pollux-14</i>	Sup3 ^e		C4804 to T	P719 to L	(-)	(-)	NODPOP+	HOM	Murray et al., 2006
<i>pollux-15</i>	S24-1B ^g		A458-C463 deletion	Q153-H154 deletion	n.d.	(+)	NODPOP+	HOM	Murray et al., 2006
<i>pollux-16</i>	SL5998-2,3,4,5,6		G496 to A	splice site	-*, -*, -*, -*	n.d., -*, n.d., n.d., n.d.	all in NODPOP	all HOM	this work
<i>pollux-17</i>	SL1657-2		G1147 to A	G301 to E	-	-	NODPOP	HOM	this work
<i>pollux-18</i>	SL0317-3 ^h		G1179 to A	G312 to S	(+*)	n.d.	NODPOP	HET	this work
<i>pollux-19ⁱ</i>	SL5821-3,5		G1644 to A	splice site	-*, *	n.d., -	both in NODPOP	both HOM	this work
<i>pollux-20</i>	SL0729-6,10		G3388 to A	W651 to stop	-*, *	-*, -	both in NODPOP	both HOM	this work
<i>pollux-21</i>	SL1075-2		G3416 to A	splice site	-	-	NODPOP	HOM	this work
<i>pollux-22</i>	SL0283-4		G4698 to A	E384 to K	n.d.	n.d.	NODPOP	HET	this work
<i>pollux-23</i>	SL4162-2		C347 to T	T116 to I	(-)	(-)	NODPOP	HOM	this work
<i>pollux-24</i>	SL1913-7,8 ^k		C1922 to T	intron 4	(+/-), (-*)	(+), n.d.	both in NODPOP	both HET	this work
<i>pollux-25</i>	SL5546-2,3		C3568 to T	intron 8	+/-*, +/-*	n.d., n.d.	both in NODPOP	both HET	this work

<i>pollux-26</i>	SL0405-4	G2837 to A	intron 6	-*	n.d.	NODPOP	HET	this work
<i>pollux-27</i>	SL0299-2	G2872 to A	intron 6	-*	n.d.	NODPOP	HOM	this work
<i>pollux-28</i>	SL0456-2 ^k	T5897 to G	intron 11	(+)	n.d.	NODPOP	HET	this work
<i>pollux-29</i>	SL0387-3	G4646 to A	L666 to L	-*	n.d.	NODPOP	HOM	this work
<i>pollux-30</i>	SL1913-8 ^l	G6023 to A	G882 to G	(-*)	n.d.	NODPOP	HET	this work

^a The *POLLUX* genomic sequence is deposited to GenBank under accession number AB162017 and is used as *Lotus japonicus* Gifu reference. Adenine of the ATG initiator codon is designated as position 1.

^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star stands for additional screening in the M₃ generation. In the nodulation phenotype "+/-" designates reduced nodule number, smaller size or white colour. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.

^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.

^d *pollux-1* and *pollux-2* are the same allele.

^e Lines contain in addition a *har1-1* mutation.

^f *pollux-12* and *pollux-19* are the same allele.

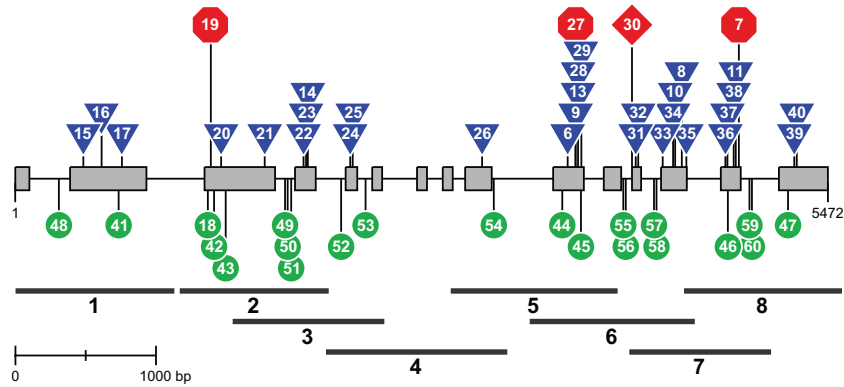
^g Line S24-1B carries the mutant alleles *pollux-15* and *symrk-28*.

^h Line SL0317-3 carries the mutant alleles *nup133-14* and *pollux-18*.

ⁱ Line SL1913-7 carries the mutant alleles *pollux-24* and *symrk-59*.

^k Line SL1913-8 carries the mutant alleles *pollux-24* and *pollux-30*.

^l Line SL0456-2 carries the mutant alleles *nfr1-5* and *pollux-28*.

SYMRK

Allele	Progenitor/ mutant line	Previous name	Genomic mutation ^a	Effect	Nodulation phenotype ^b	AM phenotype ^b	Detected by TILLING	Genotype of M ₂ plant ^c	Reference
<i>symrk-1</i>	282-287	<i>sym2</i>	~4.9 kb insertion in exon 3	frame shift	-	-			Schauser et al., 1998; Stracke et al., 2002
<i>symrk-2</i>	282-288		n.d.	n.d.	-	-			Schauser et al., 1998; Demchenko et al., 2004; Kistner et al., 2005
<i>symrk-3</i>	cac41.5	<i>sym2</i>	~5.8 kb insertion in exon 4	frame shift	-	-			Stracke et al., 2002; Demchenko et al., 2004; Kistner et al., 2005
<i>symrk-4</i>	KL481		n.d.	n.d.	-	-			Sandal et al., 2006
<i>symrk-5</i>	cac67.6		n.d.	n.d.	-	-			Sandal et al., 2006
<i>symrk-6</i>	EMS34		G3723 to A	G603 to R	-	n.d.	NODPOP+	HOM	Szczyglowski et al., 1998; Perry et al., 2003
<i>symrk-7</i>	EMS61		G4872 to A	G806 to stop	-	-			Szczyglowski et al., 1998; Stracke et al., 2002; Kistner et al., 2005
<i>symrk-8</i>	SL0160-2,3,4,5,6; SL0180-1		G4510 to A	G759 to R	-; - ⁺ ; -; - ⁺ ; + ⁺	-; n.d.; -; -; n.d.; n.d.	NODPOP; GENPOP	all HOM; HET	Perry et al., 2003; this work
<i>symrk-9</i>	SL0605 ^a -2,3		G3729 to A	G604 to R	(-), (- ⁺)	(-), n.d.	both in NODPOP	both HOM	Perry et al., 2003
<i>symrk-10</i>	SL1951- 1,2,3,4,5,6,7		G4447 to A	D738 to N	n.d., - ⁺ ; -; - ⁺ ; -; -; - ⁺	n.d., n.d., -; n.d., n.d., -; n.d.	all in NODPOP	all HOM	Perry et al., 2003; Markmann et al., 2008
<i>symrk-11</i>	SL3472-2		G4832 to A	G793 to D	- ⁺	n.d.	NODPOP	HOM	Perry et al., 2003
<i>symrk-12</i>	8-4H		insertion in 5'- UTR	n.d.	-	-			Sandal et al., 2006
<i>symrk-13</i>	S50-1 ^a		G3777 to A	A620 to T	(-)	(-)			Murray et al., 2006
<i>symrk-14</i>	B13-B ^a		C1965 to T	P386 to T	(+)	(-)			Murray et al., 2006
<i>symrk-15</i>	SL0288-3,4		G429 to A	R64 to K	+ ⁺ , +	n.d., +	both in NODPOP	HET, HOM	this work
<i>symrk-16</i>	SL1288-1		G581 to A	V103 to M	+ ⁺	n.d.	GENPOP	HET	this work
<i>symrk-17</i>	SL2677-1		G722 to A	D150 to N	+	+	GENPOP	HOM	this work
<i>symrk-18</i>	SL1990-1		C1292 to T	D209 to D	+ ⁺	n.d.	GENPOP	HET	this work
<i>symrk-19</i>	SL3461-1		G1301 to A	W212 to stop	+ ⁺	n.d.	GENPOP	HET	this work
<i>symrk-20</i>	SL1559-2		C1384 to T	P240 to L	+	n.d.	NODPOP	HET	this work
<i>symrk-21</i>	282-370M1M1		A1690 to C	Y342 to S	-	n.d.	NODPOP+	HOM	N. Sandal and J. Stougaard, pers. communication; this work
<i>symrk-22</i>	SL0478-1		G1890 to A	V361 to M	+	+	GENPOP	HOM	this work
<i>symrk-23</i>	SL1023-1		G1911 to A	E388 to K	+ ⁺	n.d.	GENPOP	HET	this work
<i>symrk-24</i>	SL3006-1		A2252 to T	I420 to L	+ ⁺	n.d.	GENPOP	HET	this work
<i>symrk-25</i>	SL5686-2		A2261 to C	S423 to R	+/-	+	NODPOP	HET	this work

<i>symrk-26</i>	SL0306-1	C3126 to T	L538 to F	++	n.d.	GENPOP	HOM	this work
<i>symrk-27</i>	SL3001-1	G3768 to T	Q617 to stop	-	-	GENPOP	HET	this work
<i>symrk-28</i>	S24-1B ^f	G3777 to A	A620 to T	n.d.	(+)	NODPOP+	HOM	Murray et al., 2006; this work
<i>symrk-29</i>	SL5510-2	G3810 to A	G631 to R	+/-*	n.d.	NODPOP	HOM	this work
<i>symrk-30</i>	SL0391-2	G4155 to T	splice site	-*	n.d.	NODPOP	HOM	Perry et al., 2003
<i>symrk-31</i>	SL3489-1	C4189 to T	P62 to L	++	n.d.	GENPOP	HOM	this work
<i>symrk-32</i>	SL1225-1	C4192 to T	T693 to I	++	n.d.	GENPOP	HET	this work
<i>symrk-33</i>	SL1060-1	C4360 to T	H709 to Y	+	+/-	GENPOP	HET	this work
<i>symrk-34</i>	SL0140-2	G4441 to A	V736 to I	+/-*	n.d.	NODPOP	HET	Perry et al., 2003
<i>symrk-35</i>	SL5073-12,13; SL5627-2,3	G4528 to A	D765 to N	-,-; -,-*	-,-; n.d.,n.d.	NODPOP; NODPOP	HOM; HOM	this work
<i>symrk-36</i>	SL3269-1	G4780 to A	E776 to K	++	n.d.	GENPOP	HOM	this work
<i>symrk-37</i>	SL1538-1	G4787 to A	S778 to N	++	n.d.	GENPOP	HET	this work
<i>symrk-38</i>	SL1849-1	G4825 to A	V791 to M	++	n.d.	GENPOP	HET	this work
<i>symrk-39</i>	SL1959-1	C5255 to T	P851 to L	++	n.d.	GENPOP	HET	this work
<i>symrk-40</i>	SL0265-3,4	G5270 to A	R856 to K	-,-*	-,-n.d.	both in NODPOP	both HOM	this work
<i>symrk-41</i>	SL0783-1	G700 to A	E142 to E	++	n.d.	GENPOP	HET	this work
<i>symrk-42</i>	SL0193-1; SL0210-1	C1335 to T	L224 to L	++;+*	n.d.;n.d.	GENPOP; GENPOP	HET; HET	this work
<i>symrk-43</i>	SL1401-1	G1421 to A	E252 to E	++	n.d.	GENPOP	HET	this work
<i>symrk-44</i>	SL0798-1	G3677 to A	L586 to L	++	n.d.	GENPOP	HOM	this work
<i>symrk-45</i>	SL0275-3	G3809 to A	Q630 to Q	++	n.d.	NODPOP	HOM	this work
<i>symrk-46</i>	SL6805-3	C4800 to T	S782 to S	-*	n.d.	NODPOP	HET	this work
<i>symrk-47</i>	SL1813-1	C5202 to T	Y633 to Y	++	n.d.	GENPOP	HET	this work
<i>symrk-48</i>	SL1824-1	C289 to T	intron 1	++	n.d.	GENPOP	HET	this work
<i>symrk-49</i>	SL0978-1	C1817 to T	intron 3	++	n.d.	GENPOP	HET	this work
<i>symrk-50</i>	SL1153-3	C1846 to T	intron 3	++	n.d.	NODPOP	HET	this work
<i>symrk-51</i>	SL0710-1	C1879 to T	intron 3	++	n.d.	GENPOP	HET	this work
<i>symrk-52</i>	SL1084-1	C2053 to T	intron 4	++	n.d.	GENPOP	HET	this work
<i>symrk-53</i>	SL2023-1	G2353 to A	intron 5	++	n.d.	GENPOP	HOM	this work
<i>symrk-54</i>	SL0470-1; SL0825-1	C3213 to T	intron 9	++;+*	n.d.;n.d.	GENPOP; GENPOP	HET; HET	this work
<i>symrk-55</i>	SL0759-2	C4083 to T	intron 11	-*	+	NODPOP	HET	this work
<i>symrk-56</i>	SL0838-1; SL1599-1	C4105 to T	intron 11	++;+	n.d.;+	GENPOP; GENPOP	HET; HET	this work
<i>symrk-57</i>	SL1532-1	G4306 to A	intron 12	++	n.d.	GENPOP	HET	this work
<i>symrk-58</i>	SL1749-1	G4308 to A	intron 12	++	n.d.	GENPOP	HET	this work
<i>symrk-59</i>	SL1913-7 ^a	G4653 to A	intron 14	(+/-)	(+)	NODPOP	HET	this work
<i>symrk-60</i>	SL1739-1, SL1846-1	C4960 to T	intron 14	++;+*	n.d.;n.d.	GENPOP; GENPOP	HET; HET	this work

^a The *SYMRK* genomic sequence is deposited to GenBank under accession number AP004579 and is used as *Lotus japonicus* MG20 reference. Adenine of the ATG initiator codon is designated as position 1.

- ^b Phenotypes that are scored only in the initial M₂ generation are denoted by *; no star denotes additional screening in the M₃ generation. In the AM phenotype "+/-" designates the appearance of balloon-like structures. Annotations in brackets indicate lines harbouring more than one mutation in nodulation genes.
- ^c Genotype of progenitor or mutant plant in the M₂ generation is given as HOM = homozygous or HET = heterozygous with respect to the mutant allele.
- ^d Lines SL0605-2,3 carry the mutant alleles *nin-11* and *symrk-9*.
- ^e Lines contain in addition a *har1-1* mutation.
- ^f Line S24-1B carries the mutant alleles *pollux-15* and *symrk-28*.
- ^g Line SL1913-7 carries the mutant alleles *pollux-24* and *symrk-59*.

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Supplemental Fig. S4. BLOSUM62 table with amino acid changes.

	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y	*
A	4	0	-2	-1	-2	0	-2	-1	-1	-1	-1	-2	-1	-1	-1	1	0	0	-3	-2	-4
C	0	9	-3	-4	-2	-3	-3	-1	-3	-1	-1	-3	-3	-3	-3	-1	-1	-1	-2	-2	-4
D	-2	-3	6	2	-3	-1	-1	-3	-1	-4	-3	1	-1	0	-2	0	-1	-3	-4	-3	-4
E	-1	-4	2	5	-3	-2	0	-3	1	-3	-2	0	-1	2	0	0	-1	-2	-3	-2	-4
F	-2	-2	-3	-3	6	-3	-1	0	-3	0	0	-3	-4	-3	-3	-2	-2	-1	1	3	-4
G	0	-3	-1	-2	-3	6	-2	-4	-2	-4	-3	0	-2	-2	-2	0	-2	-3	-2	-3	-4
H	-2	-3	-1	0	-1	-2	8	-3	-1	-3	-2	1	-2	0	0	-1	-2	-3	-2	2	-4
I	-1	-1	-3	-3	0	-4	-3	4	-3	2	1	-3	-3	-3	-3	-2	-1	3	-3	-1	-4
K	-1	-3	-1	1	-3	-2	-1	-3	5	-2	-1	0	-1	1	2	0	-1	-2	-3	-2	-4
L	-1	-1	-4	-3	0	-4	-3	2	-2	4	2	-3	-3	-2	-2	-2	-1	1	-2	-1	-4
M	-1	-1	-3	-2	0	-3	-2	1	-1	2	5	-2	-2	0	-1	-1	-1	1	-1	-1	-4
N	-2	-3	1	0	-3	0	1	-3	0	-3	-2	6	-2	0	0	1	0	-3	-4	-2	-4
P	-1	-3	-1	-1	-4	-2	-2	-3	-1	-3	-2	-2	7	-1	-2	-1	-1	-2	-4	-3	-4
Q	-1	-3	0	2	-3	-2	0	-3	1	-2	0	0	-1	5	1	0	-1	-2	-2	-1	-4
R	-1	-3	-2	0	-3	-2	0	-3	2	-2	-1	0	-2	1	5	-1	-1	-3	-3	-2	-4
S	1	-1	0	0	-2	0	-1	-2	0	-2	-1	1	-1	0	-1	4	1	-2	-3	-2	-4
T	0	-1	-1	-1	-2	-2	-2	-1	-1	-1	-1	0	-1	-1	-1	1	5	0	-2	-2	-4
V	0	-1	-3	-2	-1	-3	-3	3	-2	1	1	-3	-2	-2	-3	-2	0	4	-3	-1	-4
W	-3	-2	-4	-3	1	-2	-2	-3	-3	-2	-1	-4	-4	-2	-3	-3	-2	-3	11	2	-4
Y	-2	-2	-3	-2	3	-3	2	-1	-2	-1	-1	-2	-3	-1	-2	-2	-2	-1	2	7	-4
*	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	-4	1

The original amino acids are given in rows, the substituted amino acids in columns. The star symbol (*) designates stop codons. Changes that can be caused by EMS are indicated in colour: orange, non-synonymous exchange; red, nonsense mutation. Light green shading denotes synonymous exchanges, grey shading all additional non-synonymous changes or nonsense mutations that could be achieved by a single nucleotide exchange. Note that only a small fraction of the possible mutational spectrum is explored by EMS mutagenesis.

Supplemental Table S1. Mutation types by nucleotide change detected in GENPOP and NODPOP populations.

Change	GENPOP		NODPOP ¹		Total	
	Absolute number	Relative number	Absolute number	Relative number	Absolute number	Relative number
G to A	298	51.8%	34	72.9%	332	53.3%
C to T	264	45.8%	10	20.8%	274	44.0%
A to G	1	0.17%			1	0.16%
A to T	3	0.52%			3	0.48%
C to G	1	0.17%			1	0.16%
G to C	1	0.17%			1	0.16%
G to T	3	0.52%	1	2.1%	4	0.64%
T to C	4	0.69%			4	0.64%
T to G	1	0.17%			1	0.16%
G del.			1	2.1%	1	0.16%
C del.			1	2.1%	1	0.16%
Sum	576	100%	47	100%	623	100%

¹ Numbers for the NODPOP population are for nodulation genes only, include only potentially causative mutations in families with clear nodulation phenotype (see Supplementary Table 3 and Supplementary Figure 2), and exclude siblings.

Supplemental Table S2. Distribution of mutation types detected in nodulation genes in the complete NODPOP by TILLING.

Gene	TILLed (in bp)			Mutations detected in NODPOP by TILLING				
	Total	Coding	Non-coding	Total	Homozygous non-silent	Homozygous silent	Homozygous non-coding	Heterozygous
<i>CASTOR</i>	5575	2474	3101	13	9		1	3
<i>CCaMK</i>	3973	1641	2332	5	1		1	3
<i>CYCLOPS</i>	4214	1557	2657	3	2			1
<i>NFR1</i>	5557	1866	3691	8	4		1	3
<i>NFR5</i>	1795	1788	7	5	4			1
<i>NIN</i>	3047	2587	460	5	3			2
<i>NSP1</i>	1444	1444	0	3	1	1		1
<i>NSP2</i>	1440	1440	0	4	2			2
<i>NUP85</i>	8391	2136	6255	3	1		1	1
<i>NUP133</i>	6353	3842	2511	8	2	1		5
<i>POLLUX</i>	6535	2754	3781	22	12	1	1	8
<i>SYMRK</i>	5365	3282	2083	18	10	1		7
Sum	53,689	26,811	26,878	97	51	4	5	37

Supplemental Table S3. Alleles in nodulation genes with potentially causative mutations in the nodulation-deficient NODPOP, ordered by mutation type.

Allele	NODPOP family	Mutation	Nucleotide change
<i>nfr1-10</i>	SL1174	A ₃₅₀ to T	G ₂₉₄₁ to A
<i>castor-14</i>	SL1715	A ₇₆₀ to T	G ₉₈₇₁ to A
<i>castor-13</i>	SL0820	D ₄₄₄ to N	G ₂₆₇₂ to A
<i>symrk-10</i>	SL1951	D ₇₃₈ to N	G ₄₄₄₇ to A
<i>symrk-35</i>	SL5073	D ₇₆₅ to N	G ₄₅₂₈ to A
<i>symrk-35</i>	SL5627	D ₇₆₅ to N	G ₄₅₂₈ to A
<i>nin-11</i>	SL0605	E ₅₄₀ to K	G ₁₈₄₈ to A
<i>pollux-9</i>	SL0405	E ₈₃₀ to K	G ₅₆₃₄ to A
<i>castor-26</i>	SL6908	G ₂₇₄ to D	G ₁₀₈₈ to A
<i>symrk-11</i>	SL3472	G ₇₉₃ to D	G ₄₈₃₂ to A
<i>castor-16</i>	SL3160	G ₃₈₃ to E	G ₁₆₅₅ to A
<i>nfr1-6</i>	SL5426	G ₄₈₅ to E	G ₄₃₇₇ to A
<i>pollux-7</i>	SL1899	G ₅₃₀ to E	G ₂₃₁₃ to A
<i>pollux-17</i>	SL1657	G ₃₀₁ to E	G ₁₁₄₇ to A
<i>nfr5-8</i>	SL1719	G ₂₆₄ to R	G ₇₉₀ to A
<i>symrk-9</i>	SL0605	G ₆₀₄ to R	G ₃₇₂₉ to A
<i>symrk-29</i>	SL5510	G ₆₃₁ to R	G ₃₈₁₀ to A
<i>symrk-8</i>	SL0160	G ₇₅₉ to R	G ₄₅₁₀ to A
<i>pollux-10</i>	SL1070	G ₈₈₂ to S	G ₆₀₂₁ to A
<i>nfr1-11</i>	SL1977	Q ₃₁₆ to stop	C ₂₄₁₂ to T
<i>nfr5-3</i>	SL3257	Q ₅₅ to stop	C ₁₆₃ to T
<i>nsp2-3</i>	SL0781	Q ₂₄₄ to stop	C ₇₃₀ to T
<i>nup133-11</i>	SL5179	Q ₂₇₁ to stop	C ₁₀₉₇ to T
<i>nup133-12</i>	SL0443	Q ₁₁₄₀ to stop	C ₅₈₇₄ to T
<i>castor-17</i>	SL6812	R ₅₉₀ to H	G ₃₇₃₈ to A
<i>symrk-40</i>	SL0265	R ₈₅₆ to K	G ₅₂₇₀ to A
<i>cyclops-6</i>	SL0488	R ₇₄ to stop	C ₇₅₂ to T
<i>nup85-6</i>	SL0948	S ₂₃₅ to F	C ₂₇₀₁ to T
<i>pollux-6</i>	SL5691	S ₃₂₂ to F	C ₁₂₁₀ to T
<i>castor-15</i>	SL1966	T ₂₄₉ to I	C ₁₀₁₃ to T
<i>pollux-23</i>	SL4162	T ₁₁₆ to I	C ₃₄₇ to T
<i>nin-9</i>	SL5369	V ₂₅₈ to M	G ₁₀₀₂ to A
<i>nin-9</i>	SL5426	V ₂₅₈ to M	G ₁₀₀₂ to A
<i>castor-5</i>	SL1937	W ₄₈₃ to stop	G ₃₀₅₃ to A
<i>castor-12</i>	SL3251	W ₉₃ to stop	G ₂₇₉ to A
<i>ccamk-8</i>	SL0820	W ₃₃₆ to stop	G ₃₉₂₀ to A
<i>pollux-5</i>	SL3130	W ₃₂₀ to stop	G ₁₂₀₄ to A
<i>pollux-8</i>	SL0159	W ₇₈₀ to stop	G ₅₄₈₆ to A
<i>pollux-20</i>	SL0729	W ₆₅₁ to stop	G ₃₃₈₈ to A
<i>castor-27</i>	SL5136	Splice site	G ₁₅₀₃ to A
<i>nfr1-7</i>	SL0355	Splice site	G ₅₃₃₁ to A
<i>pollux-16</i>	SL5998	Splice site	G ₄₉₆ to A
<i>pollux-19</i>	SL5821	Splice site	G ₁₆₄₄ to A
<i>pollux-21</i>	SL1075	Splice site	G ₃₄₁₆ to A
<i>symrk-30</i>	SL0391	Splice site	G ₄₁₅₅ to T
<i>nfr5-6</i>	SL5426	Frame shift	C ₂₃₇ del.
<i>cyclops-5</i>	SL1347	Frame shift	G ₃₅₀₈ del.

Supplemental Table S4. Expected and observed occurrence of non-synonymous and nonsense amino acid exchanges in EMS alleles in different populations.

Amino acid	GENPOP ¹		NODPOP ²		RPM1 ³	
	Expected	Observed	Expected	Observed	Expected	Observed
A	15.4	11	5.6	2	6.3	4
C	1.6	0	0.8	0	0.9	0
D	6.5	12	2.3	4	3.0	0
E	7.5	10	2.9	2	4.6	4
G	16.0	20	5.3	11	6.5	13
H	2.7	2	0.9	0	1.7	0
L	4.6	6	1.7	0	3.3	7
M	2.5	0	0.9	0	2.2	0
P	10.5	12	3.7	0	4.2	8
Q	3.6	2	1.6	5	2.1	3
R	8.4	5	3.0	3	5.7	2
S	6.5	5	4.3	2	5.1	5
T	5.6	2	2.1	1	2.9	2
V	7.7	9	2.7	2	4.1	2
W	3.1	4	1.2	6	2.4	5
Total	102	102	39	39	55	55

¹ Numbers for the GENPOP population are from a total of 11,718 bp from 14 genes with a variety of functions that were screened on GENPOP for mutations.

² Numbers for the NODPOP population are for potentially causative non-synonymous and nonsense mutations in nodulation genes only (see Supplementary Table 3).

³ Numbers for RPM1 are from Tornero *et al.* (2002).