

Further information on Mutant Finder Database

This database contains information from a four-year programme screening for *Lotus japonicus* 'Gifu' mutants. Plants from an initial set of ca. 2500 families were screened for nodulation phenotypes. They were then potted on, grown for at least a month and screened for morphological characters. Up to thirty seed were sown per family. Single plants from each family were also retained for DNA preparation to contribute to a TILLING population. Seed was collected in bulk from families. Plants from a further ca. 1500 families were screened in the second year for nodulation characteristics and for starch accumulation and breakdown. Again single plants from each family were retained for DNA preparation.

For the nodulation screen, all plants were visually inspected ca. 6 weeks following inoculation with *Mesorhizobium loti* under nitrogen-limiting conditions for the occurrence of root nodules, and mutants with either no nodules, or small white nodules as well as plants with a reduced or increased nodule number (supernodulating mutants) were isolated (see table below). Furthermore, the initial 2415 plant families were screened for morphological characteristics such as abnormal root branching patterns (root mutants) leaf shape (leaf mutants), and abnormal growth habit (dwarf and stature mutants). All plants were allowed to mature, and were screened after 8-12 weeks for the occurrence of mutants affected in flower development. All mutants were grown in a glasshouse and seed was collected individually. All remaining plants from the initial 2415 families were planted in the field, and seed was collected in bulk so that each seed pack contained seeds from only one family. After 8-12 weeks these field plants were also phenotypically screened for leaf, stature and flower development mutants. This seed was collected for the purpose of future forward screens and as a backup for the isolation of mutant alleles known to segregate in a particular family. In the second year, as well as screening again for nodulation mutants, 1428 families were screened for starch mutants using iodine staining of cleared leaflets at the beginning and at the end of the day to uncover both accumulation and breakdown mutants.

One problem associated with EMS mutagenesis is the high frequency of infertile plants not only in the M1 but also in subsequent generations. Consequently, several of the phenotypically interesting M2 mutants were infertile. To increase the chances of recovering mutant alleles represented in the population of developmental mutants we harvested seed from all fertile M2 siblings. However, the M1 plant represents a mosaic of differentially mutagenised cells as mutagenesis was performed at the embryo stage. More than one cell of the embryo gives rise to the germline, and differentially mutagenised sectors of the plant might produce different amounts of seed. Some alleles, therefore, may be represented in the M2 progeny well below the 75% expected for a Mendelian allele. In several families very few M2 individuals matured and set seed, which reduced the chances of recovering a particular mutant allele.

Plants in the database are labelled xxxx-y where x represents the M2 family number and y the individual plant. Plants with the same M2 number, therefore, will be siblings. Not all plants have been photographed and the photograph present usually represents the first phenotypic category in which the mutant was placed. Thus for a mutant with an abnormal leaf, which later shows a flower or fruit phenotype, only the leaf morphology has been recorded. A full list of phenotypes is shown in the table below. The numbers in the table represent the numbers of plants bearing a particular phenotype, *but* a single plant may also be recorded more than once if it was scored for more than one phenotype. Hence the numbers are not additive.

The database also has the capacity to contain information on mutations in genes discovered in our TILLING programme. At present only one gene - the test sequence - is included, but we hope to add mutants for others as we discover them.

The population is a finite resource and currently is run on limited funding. We ask that, should we provide material to you, you return seed to us of the subsequent generation.

Category	Sub category	Sub category option	Number of plants	Category	Sub category	Sub category option	Number of plants
Architecture	Shape	Bushy	54	Leaf	Appearance	spotted	55
Architecture	Shape	Single stem	35	Leaf	Appearance	variegated	31
Architecture	Size	Compact	7	Leaf	Colour	dark green	63
Architecture	Size	Giant	1	Leaf	Colour	lime green	149
Architecture	Size	Miniature	49	Leaf	Colour	pale green	140
Architecture	Stature	erect	25	Leaf	Colour	veins/lamina contrast	13
Architecture	Stature	horizontal (forced)	18	Leaf	Colour	yellow	59
Flower	Colour	Other	1	Leaf	Colour	yellow at nod screen	130
Flower	Colour	Pale yellow	1	Leaf	Colour	yellow when young	26
Flower	Colour	white	0	Leaf	Leaf x,y	broad	9
Flower	Morphology	abnormal all	5	Leaf	Leaf x,y	narrow	275
Flower	Morphology	abnormal keel	0	Leaf	Leaf x,y	pinched	12
Flower	Morphology	abnormal standard	2	Leaf	Leaf x,y	rounded	24
Flower	Morphology	abnormal wing	3	Leaf	Leaf x,y	thick	23
Flower	Morphology	veins pale/absent	10	Leaf	Leaf x,y	very narrow	42
Flower	Reproductive organs	abnormal anthers	1	Leaf	Leaflets	fewer (than 5)	8
Flower	Reproductive organs	abnormal both	1	Leaf	Leaflets	more (than 5)	0
Flower	Reproductive organs	abnormal stigma	0	Leaf	Leaflets	normal number	0
Flower	Reproductive organs	no flowers	4	Leaf	Leaflets	single	0
Flower	Size	Absent	411	Leaf	Leaflets	variable number	8
Flower	Size	Large	0	Leaf	Shape	crinkled	185
Flower	Size	Small	2	Leaf	Shape	crinkled and downcurled"	34
Flower	Size	Tiny	0	Leaf	Shape	distorted	2
Fruit	Fertility	fertile	0	Leaf	Shape	downcurling	110
Fruit	Fertility	infertile	540	Leaf	Shape	margins raised	118
Fruit	Fertility	low fertility	134	Leaf	Shape	twisted	3
Fruit	Pod Colour	green	0	Leaf	Shape	upcurling	17
Fruit	Pod Colour	mottled	0	Leaf	Size	large	11
Fruit	Pod Colour	purple	0	Leaf	Size	normal	0
Fruit	Pod Colour	yellow	0	Leaf	Size	small	485
Fruit	Pod Shape	curved	6	Leaf	Size	tiny	127
Fruit	Pod Shape	distorted	1	Nodule	Colour	brown	2
Fruit	Pod Shape	long	0	Nodule	Colour	green	1
Fruit	Pod Shape	other	0	Nodule	Colour	white	178
Fruit	Pod Shape	short	87	Nodule	Colour	yellow	4
Fruit	Seeds	abnormal (e.g.colour/shape)	1	Nodule	Number	few	57
Fruit	Seeds	absent	0	Nodule	Number	many (super, hyper)	8
Fruit	Seeds	few	6	Nodule	Number	mixed	0
Leaf	Appearance	flecked	28	Nodule	Number	none	207
Leaf	Appearance	mottled	89	Nodule	Size	bump	2

Category	Sub category	Sub category option	Number of plants
Nodule	Size	giant	9
Nodule	Size	small	159
Product	Starch	Biosynthesis	110
Product	Starch	Breakdown	73
Root	Colour	brown	1
Root	Colour	transparent	3
Root	Colour	yellow	2
Root	Length	long	1
Root	Length	other	1
Root	Length	short	21
Root	Length	stubby	1
Root	Morphology	no hairs	0
Root	Morphology	no laterals	0
Root	Morphology	other	5
Stem	Diameter	thick	36
Stem	Diameter	thin	16
Stem	Length	dwarf (1/4-2/3 normal)	333
Stem	Length	extreme dwarf	145
Stem	Length	tall (1/2 again upwards)	0
Stem	Shape	angled	3
Stem	Shape	stiff	4
Stem	Shape	twisted	1

Morphological, symbiotic and metabolic mutants of *L. japonicus* ‘Gifu’

Mutants in categories architecture, flower, leaf, root, and stem originate from scoring an initial 2,415 M2 families (28,500 M2 plants). A total of 3843 M2 families (45,600 plants) were screened for nodulation mutants, and mutants with either no nodules, or small white nodules as well as plants with a reduced or increased nodule number (supernodulating mutants) were isolated. 1428 M2 families (17,100 plants) were screened for starch biosynthesis and breakdown. The numbers recorded represent characters scored on individual plants and thus may be sibling aggregates. Furthermore, a single plant may be altered in more than one character and will be recorded in more than one section.

Further information can be found at:

<http://www.jic.bbsrc.ac.uk/staff/trevor-wang/index.htm>

<http://www.jic.bbsrc.ac.uk/sainsbury-lab/martin-parniske/homepage.htm>

Reference

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.