

Lotus research at the John Innes Centre (JIC) and Sainsbury Laboratory (SL)

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The John Innes Centre has a long and pre-eminent history of research on legumes based around the science of nitrogen fixation and the genetics and molecular biology of peas (Casey and Davies, 1993). More lately, research on legumes worldwide has focussed on the two models amenable to modern genomics analysis, *Medicago truncatula* (www.medicago.org) and *Lotus japonicus* (www.lotusjaponicus.org). Both the John Innes and Sainsbury Laboratory have turned their attention towards these models as well.

At our site on the [Norwich Research Park](#), research on *Lotus* has concentrated on symbioses, both with rhizobium to form nodules and with fungi to make arbuscular mycorrhizae. Much effort, however, has also been made to develop platforms to accelerate our own research and to help the community. A common component to both the symbioses is signalling and extensive work is carried out studying genes involved in the plant signalling pathways that are involved, especially in the overlap between the two (Kistner and Parniske, 2002). Three groups are involved directly with *Lotus*: Martin Parniske's (SL), Trevor Wang's (Metabolic Biology, JIC) and Allan Downie's (Molecular Microbiology, JIC) and collaborations exist between all three groups (e.g. Stracke *et al.*, 2002). In the [Parniske](#) group, the early signalling events in arbuscular mycorrhizal interactions are the most important, particularly those that are common to both symbioses (Downie and Parniske, 2002). In the [Downie](#) group, the centre of attention is calcium spiking (and its role in nodulation signalling; Oldroyd and Downie, 2004), and mutants affected in early stages of infection thread growth. The [Wang](#) group is focused on later metabolic events, especially carbon partitioning enzymes such as sucrose synthase (Craig *et al.*, 1999).

Trevor Wang and Martin Parniske have collaborated on an exciting new development for legumes – TILLING (Targeting Induced Local Lesions IN Genomes; Colbert *et al.*, 2001). This technology was pioneered in plants by the Henikoff group in Seattle. We have developed a similar platform for *Lotus japonicus* over the last three years (Perry *et al.*, 2003). We set up an ethylmethane sulphonate (EMS) mutation programme to generate a population of about 5000 M2 families that would be used in both forward screens and to collect DNA samples. The initial ca. 40000 plants were screened for a large number of different phenotypes. A further group of about 1400 M2 families was screened for starch mutants to use in research on sugar metabolism (<http://www.jic.bbsrc.ac.uk/staff/trevor-wang/starchscreen.ppt>). This collection has been entered onto a web-accessible database for people to browse and order mutants (<http://data.jic.bbsrc.ac.uk/cgi-bin/lotusjaponicus/>). In total more than 60000 M2 individuals have been screened for nodulation-defective mutants. Each M2 family had one

representative that was used to collect DNA and this DNA has been employed subsequently for developing the reverse genetic, TILLING, tool.

TILLING allows you to identify plants bearing a mutation in your gene of choice. It relies on the ability of the CEL1 endonuclease to detect mismatches between normal and mutant DNA strands when they are annealed. CEL1, which can be isolated readily from celery, permits the detection of single point mutations of the type induced by chemicals, such as EMS, that are conventionally used in mutation breeding programmes.

Using ‘[Coddle](#)’ a bioinformatics tool developed by the Seattle groups, regions of your favourite gene can be selected where it is more likely that a mutation will cause a change in the corresponding protein’s activity. DNA primers can then be engineered to amplify only that portion of the gene. The DNA from the M2 individuals represents the whole spectrum of variation in the population and is mixed with DNA from normal plants. Special fluorescently-labelled DNA ‘primers’ are used to amplify specific regions of the known gene in which a mutant is desired. The amplified DNA is then heated and cooled to separate and re-anneal the strands. The DNA is then cleaved with CEL1 at the mismatch between the normal strand and the mutant strand. The strands are then separated on a DNA sequencing gel. Since the enzyme can only cleave in a single direction, the two cleaved strands have reciprocal sizes that can easily be recognised on the gel from their fluorescence and position thus identifying the DNA sample and the corresponding plant with a mutation in the gene of interest. This plant is then used to study the function of the gene or it can be used directly and incorporated into a breeding programme. The whole process can be viewed in cartoon format at <http://www.lotusjaponicus.org/Illustration.html> (remember to have sound on!).

Collaborations like this one underpin much of the work at the Centre and have led to a successful bid for an EU Marie Curie Research and Training Network, INTEGRAL (www.lotusjaponicus.org/integral), coordinated by Martin Parniske. Its predecessor, LOTUS, finished this year and will culminate in the production of ‘The *Lotus japonicus* Handbook’ that details background information and techniques to aid research on this model plant. It is hoped that the volume will be published next year.

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