

Fine mapping the *afila* locus in *Pisum sativum*

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The aim of this project is to isolate the *Afila* (*Af*) gene from pea. To achieve this we developed a strategy based on the knowledge that the pea genome has a very similar gene order to *Medicago truncatula*. (1,2) This means that genetic mapping in pea using gene based markers can relate a fine scale genetic map in pea to the known sequence of *Medicago truncatula*.



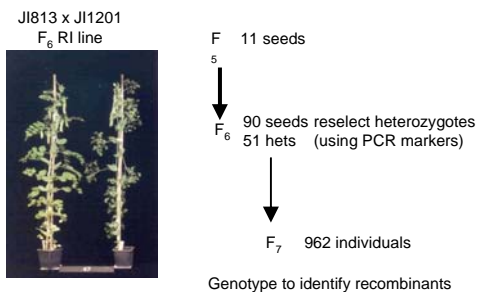
Af/Af **af/af**



lodging

The *Afila* gene has a major impact on leaf form in pea, and the *afila* mutation is widely used in pea cultivars as its system of branched tendrils has several beneficial effects such as increased resistance to lodging.

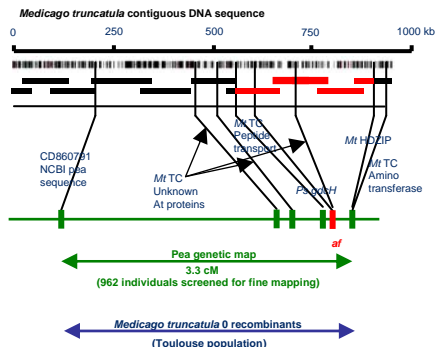
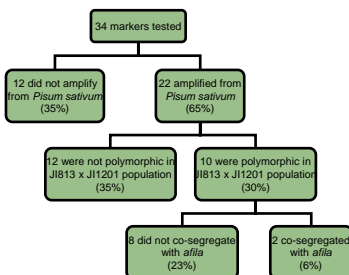
Developing a mapping population



Seeding the fine mapping strategy

A probe based on the pea *Gdch* coding sequence identified a marker which co-segregated with *afila* in the J1813xJ11201 RI population. This was used to screen part of a *Medicago truncatula* BAC library.(3) Six positive clones were obtained and these enabled us to identify a contig from BAC fingerprint data. Two BACs in the contig had been sequenced at Oklahoma, and a further eight BACs were sequenced as part of the GLIP contribution to the *Medicago* genome sequencing project. Primer pairs were designed from predicted ORFs within these BACs and used for further mapping.

Progress of the fine mapping



The closest *afila* flanking markers identify a 390kb *Medicago* contig on four BAC clones. IMGAG ORF predictions* identify around seventy potential genes in this region.

*TIGR http://www.tigr.org/tigr-scripts/medicago/IMGAG/imgag_annotator.pl?browse=1 and UrMeLDB <http://mips.gsf.de/proj/plant/jstf/medi/index.jsp>



Wild type **11/47 *afila***

Pairs of isogenic wild type and mutant *afila* lines are being used to look for allelic differences in candidate sequences. These include the 11/47 *afila* allele which has a novel phenotype.

Candidate Gene Approach

Initially we investigated an HDZIP sequence as a possible interactor with *Tendrils-less*, but this showed no difference in expression between wild type and mutant *afila* lines. It also turned out not to segregate with *afila* in one line of our extended population. Now we are looking at other transcription factors.

