TILLING for a Reduced Allergen Peanut

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Peanut (*Arachis hypogaea* L.) is a crop with little genetic diversity at the DNA level and limited resources for disease resistance and agronomic traits within the species. Wild relatives contain considerable genetic diversity, although they are diploid Since peanut is an allotetraploid, it contains four copies of any one gene. Two of these copies belong to one of the peanut's progenitor genomes (probably *A. duranensis* – A-genome) and two belong to the other progenitor genome (probably *A. ipaensis* – B-genome). They do not normally exchange with each other because of the lack of chromosome pairing . We have used funding from the Georgia Peanut Commission to characterize the DNA sequence of the multiple copies of two allergen genes, *ara h 2* and *ara h 6*, and to develop TILLING populations. There are two copies of *ara h 2* in the peanut genome and gene-specific primers are being used for TILLING. This approach simplifies the ongoing TILLING project in polyploid peanut.

TILLING, Targeting Induced Local Lesions IN Genomes (McCallum et al. 2000. Plant Physiol. 123:439) yields an allelic series of mutations that can be screened using an inexpensive and reliable high throughput methodology. It has proved to be valuable for recovering mutations in known genes of *Arabidopsis thaliana*, a model for plant genomics. The method of TILLING has allowed us to identify mutations in *ara h 2.01* in cultivated peanut from an EMS-mutagenized population. One mutant individual (37-4) was confirmed by sequencing to contain two mutations, a missense mutation at position 164 and a silent mutation at position 192. The substitution at position 164 induced an amino acid change at position 55. This change affects the allergenic epitope #5 (KIQRDEDSYE/ KIQ**H**DEDSYE). A second mutant 20-6 shows a missense mutation at position 145, which induces a change at position 49 at the protein level affecting the epitope #4 (LRPCEQHLMQ/ LRPCEQH**F**MQ). To corroborate the presence of the mutations in the subsequent generation, M3 samples from mutants 20-6 and 37-4 were analyzed. All M3 plants showed the mutations. Seed proteins from 20-6 and 37-4 mutants were evaluated for Ara h 2.01 content by western blotting with anti-Ara h 2 chicken antibody. The samples were normalized for Ara h 2.01 and were analyzed for IgE-binding capacity with serum from peanut-allergic individuals. No differences in IgE binding were observed among these isoforms After screening 352 individuals from the DES-mutagenized population for both *ara h 2.01* and *ara h 2.02*, three putative mutants were identified in pools, although these could not be verified by screening individuals. Additional pools will need to be screened before concluding that the DES treatment was not sufficiently mutagenic to use in TILLING. Also, 376 individuals from a second EMS-mutagenized population were tested with two putative mutants identified in pools. One mutant (34-2) was verified to have a nucleotide change (G/A) at position 192.

We also have conducted Ecotilling on 30 accessions of the diploid peanut relative *A. duranensis*, and discovered variation for *ara d 2.01* in 7 of the accessions. The sequences encoding the mature proteins of the wild-type and variants Q47E, S73T and D158N were cloned in the expression vector pET-24b and sequenced. The recombinant proteins were purified and analyzed immunologically. Recombinant proteins showed a different IgE reaction than native proteins and these apparent differences are the subject of additional experimentation.

Laura Ramos, the postdoc who initiated the peanut TILLING project moved to another TILLING laboratory (soybean) in November. She has been replaced by Joseph Knoll, a postdoc who began working on the project in January.