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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT
RESEARCH INITIATIVE
QUARTERLY REPORT FOR WORK
DONE UNDER RESEARCH AGREEMENT

Final Report

INSTITUTION: University of Georgia

PROJECT TITLE: Enhancing a TILLING resource for peanut

RES. AGR. NO.: #239

PROJECT LEADER: P. Ozias-Akins

GACCP Control NO.: 4-896-653-5

EXPIRATION DATE: June 30, 2009

NPB CONTACT: Marie Fenn or M Mehok

NPB Control NO.: 239

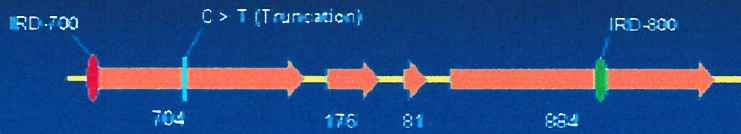
REPORT OF PROGRESS:

The method of TILLING has successfully identified mutations in *ara h 2.01*, *ara h 2.02*, and two *ara h 1* genes in cultivated peanut from EMS-mutagenized populations consisting of ~3000 M2 lines. After screening 352 individuals from the DES-mutagenized population for all four allergen genes under study, no mutations were confirmed. The first mutants for *ara h 1 β* and *ara h 1 α* were recently detected on TILLING gels using DNA from a new EMS-mutagenized population of 384 M2 plants. This would be the third population tested that has yielded one or more mutants. The sequences of these mutants have been verified. One mutation in *ara h 1 β* introduces a premature stop codon in the first exon, which should result in a significantly truncated protein. M3 seeds from this mutant were planted in the greenhouse, and the presence of the mutation was verified in six of seven plants, though none were found to be homozygous for the mutation. M4 seeds have been harvested and a cotyledon chip from each will be analyzed for protein changes while planting the remainder of the seed for germination. An interesting mutation that alters the start codon of *Ara h 2.02* also has the potential to eliminate this protein. The M2 plant produced only 2 seeds; therefore, we do not know if this mutation will be recovered in progeny.

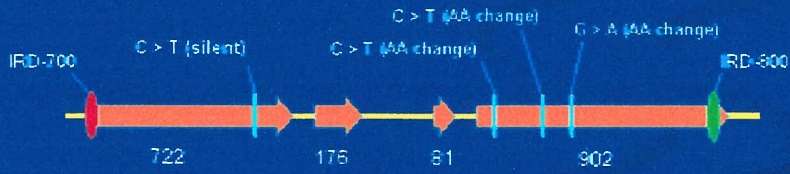
In summary, > 3000 mutant lines already have been screened, which resulted in the identification of 12 confirmed *ara h 2* and five confirmed *ara h 1* mutations (see figures below). All of the confirmed mutations are missense or silent except for one confirmed *ara h 1* mutation which is nonsense and should result in a knockout of expression. One missense mutation, however, occurs in the start codon of *Ara h 2.02* which is likely to produce an aberrant protein that should be degraded. Further analysis of homozygous lines, if they can be recovered, will be necessary before the effect of mutations on protein products can be determined.

The population of M1 lines was more than doubled in 2008 with additional EMS treatments and cultivation of material in the field. M2 seed (~7000 lines) were harvested. Of these, ~2000 were planted in the field in spring 2009 and plant material was harvested for DNA extraction and future TILLING.

Ara h 1 Mutations



Ara h 1β (A genome)

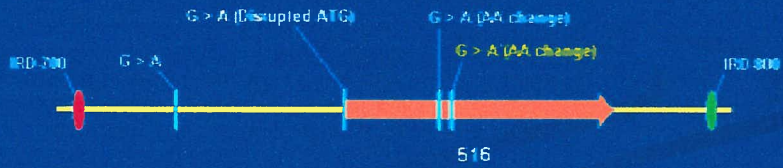


Ara h 1α (B genome)

Ara h 2 Mutations



Ara h 2.01 (A genome)



Ara h 2.02 (B genome)