

NATIONAL PEANUT BOARD/SOUTHEAST PEANUT

Final Report
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RESEARCH INITIATIVE
FINAL REPORT FOR WORK
DONE UNDER RESEARCH AGREEMENT

INSTITUTION: University of Georgia

PROJECT TITLE: Development of a Tilling Resource for Peanut

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GACCP Control NO.: 4-878

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REPORT OF PROGRESS:

The method of TILLING has successfully identified mutations in *ara h 2.01* in cultivated peanut from EMS-mutagenized populations. Two mutants have missense mutations at positions 145 and 164, respectively. The substitution at position 164 induced an amino acid change at position 55. This change affects the allergenic epitope #5 (KIQRDEDSYE/ KIQHDEDSYE). A second mutant shows a missense mutation at position 145, which induces a change at position 49 at the protein level affecting the epitope #4 (LRPCEQHLMQ/ LRPCEQHFMQ). To corroborate the presence of the mutations in the subsequent generation, M3 samples from the two were analyzed. All M3 plants showed the mutations. IgE binding to the variant proteins appears to be unchanged. Also, 376 individuals from a second EMS-mutagenized population were tested with two putative mutants identified in pools, although only one of these could be verified as having a silent mutation (no amino acid change) at position 192. Approximately 1000 additional M1 mutants were harvested from the field. DNA is being extracted from 384 of the M2 lines growing in the greenhouse. Materials have been purchased for DNA extraction and TILLING gels to survey these individuals.

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 have been purified and analyzed immunologically. Although the recombinant proteins show no variation in IgE binding between wild-type and mutants, the native S73T mutation consistently shows slightly reduced binding to IgE antibodies from some sera.

Publications of both the TILLING and Ecotilling work are in preparation.