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

Final Report
Jun. 30, 2012

INSTITUTION: University of Georgia

PROJECT TITLE: **Genetic Mapping and Mutation Discovery for Disease Resistance**

RES. AGR. NO.:
GACCP Control NO.:

PROJECT LEADER: P. Ozias-Akins

EXPIRATION DATE: Jun. 30, 2012

NPB CONTACT:
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REPORT OF PROGRESS:

1. Generate a molecular map of the 'Gregory' x 'Tifguard' population using simple-sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers and determine which regions of the genome are associated with leaf spot resistance/susceptibility.

SNP and SSR polymorphisms have been identified by genotyping the parents and DNA from the population has been extracted for genotyping. Only a small number of currently identified SNPs (38) are polymorphic between these two parents. A set of 331 SSR primer pairs has been screened on the parents out of which 81 (24.5%) reveal polymorphic markers. Leaf spot disease ratings according to the Florida 1-10 scale were collected during the summer of 2011 on three replications of each line in the population. The lateral stem image analysis assay of samples taken from the same plots included % diseased area, infection frequency, and % defoliation all of which were significantly correlated with the Florida 1-10 scale rating but enabled better quantification. Components of leaf spot disease were assessed in a detached leaf experiment where segregation was observed for latent period, sporulation, and percent area of leaf with lesions. Screening of the population with polymorphic SSR and SNP markers is in progress. A genetic map will be constructed once these data are completed.

2. Identify SSR and SNP polymorphisms that distinguish NC3033 and SPT 06-06 from Florida-07, parents of new RIL populations segregating for leaf spot resistance.

Only a few SNP polymorphisms have been identified. Up to now 120 SSR primer pairs show polymorphic markers for Tifrunner and NC3033 while 75 are polymorphic between SPT-06-06 and Florida-07. A sequence-based approach to identify additional SNP markers is in progress.

3. Implement a sequence-based approach to screen the TILLING population for mutations in a suite of genes that have been shown to respond to drought, disease, or other stresses and test the feasibility of using zinc-finger mutagenesis to target specific genes. Ten genes (Ara h 1.01, Ara h 1.02, Ara h 2.01, Ara h 2.02, FAD2A, FAD2B, LOX3 (2 copies co-amplified), SADA, SADB) were selected to test sequencing by TILLING. 768 M2 DNAs were amplified for each, pooled, and barcoded for sequencing. Each individual is present in two pools to allow confirmation of a sequence difference. Amplicons from Tifrunner were sequenced to serve as the reference against which pooled sequences are being aligned. Sequencing of the pooled, indexed amplicons has been completed and pools were demultiplexed. 367,821,228 total reads were obtained from one Illumina Hi-Seq lane of which 240,023,204 were high-quality and 212,071,861 were full-length. The average read length was 91 nt for a total of 21.8 Gb sequence. Filtering parameters have been determined that allowed identification of known mutations within the samples. Using these parameters, 5-12 times the number of previously identified mutants was indicated, and validation of new mutations is in progress.