## LONG-TERM GERMPLASM ENHANCEMENT AND DEVELOPMENT OF DNA MOLECULAR MARKER RESOURCES FOR PEANUT

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Enhancing genetic diversity is critical for ensuring the continued success of plant breeding programs. Making crosses between genetically similar individuals limits potential genetic gain and a narrow genetic base makes a crop vulnerable to threats such as new diseases, pests, or climatic conditions. To maintain market competitiveness and sustainability of peanut farming in Georgia, it is prudent to increase genetic diversity and incorporate novel alleles into the breeding pipeline. The objectives of this ongoing project are to (1) evaluate genetic distance among cultivars, breeding lines, and external germplasm to assist in parental selection and to make more effective cross combination decisions; (2) explore and develop new germplasm resources to incorporate novel alleles for important traits; and (3) develop mapping populations to identify new DNA markers for genes controlling disease resistance, agronomic, or quality traits important to Georgia peanut growers.

Using tGBS, a DNA sequencing method, cultivars released by the University of Georgia were assessed for their genetic similarity. Single nucleotide polymorphisms (SNP), which are differences in the DNA sequences were identified and aligned to the recently published *A. hypogaea* genome sequence. The analysis identified a total of 26,942 SNPs that were well-distributed across the 20 A. hypogaea chromosome pairs (Fig. 1). Thirty-two cultivars, representing the output of the UGA peanut breeding program since its inception, were selected for the first phase of this project. Tissue samples were collected from 12 individual plants per cultivar to determine within-line variation as well as among lines. Within-cultivar genetic variation was low, with most of the individual plants within a cultivar similar by >98%, indicating very little outcrossing, minimal seed mixtures, and excellent overall genetic purity of breeder-maintained seed. Genetic similarity between cultivars ranged from as low as 57.7% (Dixie Spanish vs. Georgia 119-20) to 99.2% (Virginia Bunch G2 vs. Virginia Bunch 67), with an average pairwise genetic distance of 87.6% among all 32 cultivars. Breeding has increased genetic diversity within the program over time compared to the early cultivar releases which were the product of recurrent selection programs rather than hybridization breeding programs as they are today. An unrooted phylogenetic tree (Fig. 2) provides a visualization of the genetic relationships among these 32 UGA cultivars (GA). GA-06G and GA Greener are full-sibs, that is, they come from a cross of the same parents and their genetic similarity of 97.6% is indicative of this relationship. However, the pedigree of two lines does not always reflect their true genetic distance. For instance, GA-10T is the product of a cross between GA-02C and GA-01R. Pedigree-based estimation would lead one to believe GA-10T should be equally similar to its parents, GA-02C and GA-01R. However, tGBS-based distance analysis reveals that GA-10T is 97.1% similar to GA-01R, and 85.4% similar to GA-02C.

We are currently developing a diverse set of populations for phenotypic evaluation and selection to improve resistance for Late Leaf Spot (LLS) and Tomato Spotted Wilt Virus (TSWV). Some of the parents we are using include WS-16, an *A. cardenasii* introgression line with good resistance to LLS but poor agronomic characteristics; NC 94022, an *A. hypogaea* subsp. *hypogaea* var. *hirsuta* line with high levels of resistance to TSWV with which we hope to improve upon our high levels of resistance; and TxAG-6, a synthetic tetraploid, tri-species hybrid with resistance to root knot nematode (RKN) and LLS.

Based upon a recent bulked-segregant analysis (BSA) conducted on a population segregating for bunch growth habit, we identified a region that likely contains a gene or genes controlling bunch growth habit. We are using GPC funds to further validate the results and develop diagnostic DNA markers. Additionally, we have initiated several quantitative trait locus (QTL) mapping populations, the first of which will be planted in the field this year. We will be looking at disease resistance, several growthrelated traits, and seed traits. We are especially interested in identifying genomic regions for resistance to Leaf Scorch or Pepper Spot, which is a disease that can reach fatal levels in the greenhouse. Figure 1. SNP density revealed by tGBS among peanut cultivars released by UGA from 1938 to 2018



Figure 2. Unrooted phylogenetic tree indicating tGBS-based genetic distance of peanut cultivars released by UGA from 1938 to 2018.

