Annual report + Summary

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## I. Genetic fine-mapping the major resistance gene controlling the TSWV in peanut

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## III. Project progress report for funding period 05-20-2015~06-22-2016

The **specific research objectives** are to 1) develop a large population with more than 1000 lines segregating on the major resistance gene; 2) develop additional simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers at the major resistance QTL interval to fine map the major resistance gene.

To develop the segregating population, the two flanking SSR markers of the TSWV resistance QTL was used to genotype the individuals in aF2:5 populations derived from a cross between UF113, the resistant line and Georgia Valencia, the susceptible line. 23 individuals in the F2:5 population showing heterozygous genotypes at both flanking SSR markers. These 23 F2:5 individual were harvest separately and all of their seeds planted in 2015 and grown into F2:6 families. In total 23 F2:6 families had 2430 plants. The TSWV infection level of these 2430 plants was scored in the field. In total, 138 plants showed medium infection and 84 plants showed severe infection. The leave samples of the 2430 individual plants were collected and DNA samples were extracted from them for genotyping with newly developed SSR and SNP markers.

To develop new SNP markers, we have applied genotyping by sequencing (GBS) of two parental lines (Florida-EP$^{TM}$ '113' and Georgia Valencia) plus 10 $F_6$ lines samples. Among the 10 $F_6$ lines, five lines consistently showed disease resistance for three years and the other five lines showed susceptibility. The GBS experiment generated a total of 1,856,429 sequences reads and 7,972 raw SNPs were called by Tassel-GBS pipeline. After removing the SNPs with high level of missing data (>25%), a total of 2,670 SNPs were identified. Manhattan plot depicted the association mapping results, which indicated seven SNPs were located at the major QTL region on chromosome A01 associated with spotted wilt disease resistance (Figure 1). These SNP markers after validation will be utilized to genotype the fine mapping population above.

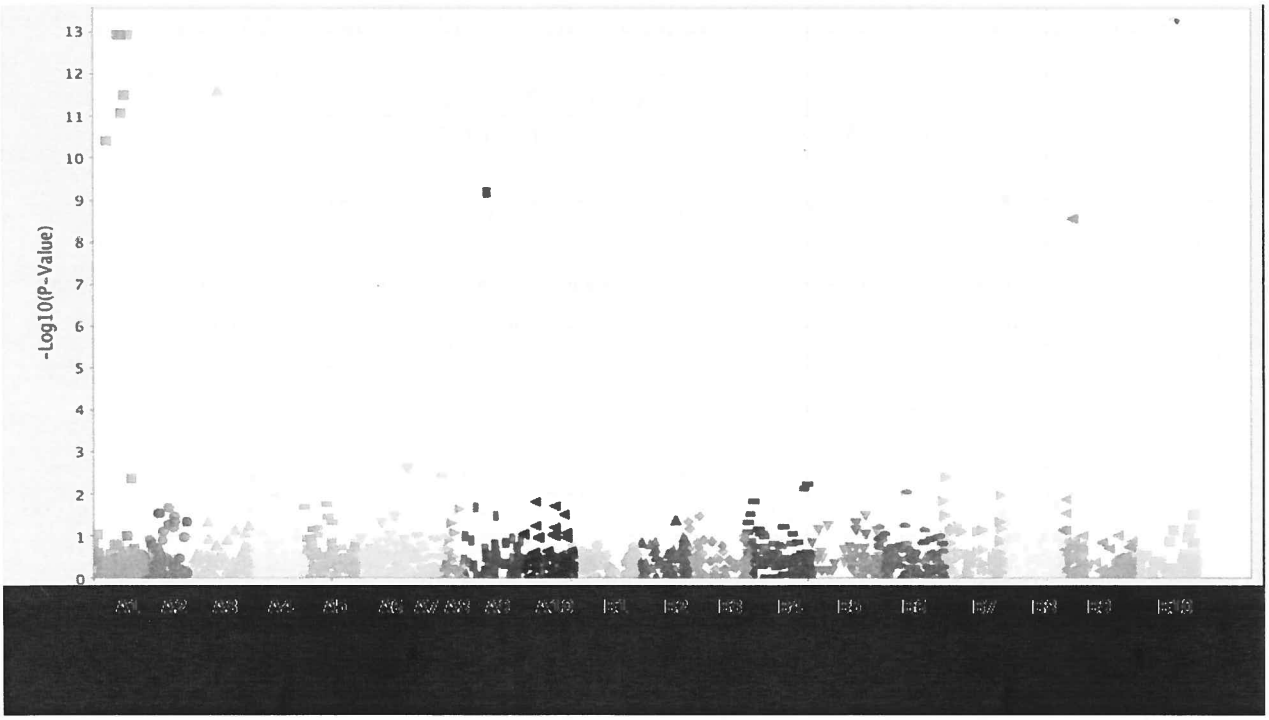

Figure 1. Manhattan plot showing the associations between SNPs and spotted wilt resistance on different peanut chromosomes.

To increase the mapping resolution on chromosome A01, an additional 154 SSR markers from latest literature on chromosome A01 were recruited for polymorphism screening using the parental lines. Overall 29 polymorphic SSR markers on A01 were screened, which have been further used to genotype the $F_2$ population of 163 individuals to generate a genetic map with narrower distance between the flanking markers.

Linkage analysis revealed that out of 29 polymorphic SSR markers 23 markers were mapped on one linkage group. The genetic distance was 157.80 cM (Figure 2). Out of 23 linked SSR markers, 19 markers can be aligned to the reference genome on chromosome A01. The physical positions of seven SNP markers from GBS data analysis above were also indicated on the physical map (Figure 2). On the physical map, the top marker aligned to A01 was AhTE0369 and the position was at 5.7 megabase. The bottom marker was AHGS1351 and the position was at 105.1 megabase. A high collinearity between the genetic and physical maps was observed (Figure 2, Table 1).

Table 1. The positions of A01 markers on physical (Mb) and linkage map (cM) in cross between Florida-EP™ '113' and Georgia Valencia peanut cultivars.

| Marker name | Physaical map (Mb) | Linkage map (cM) |
|---|---|---|
| AhTE0369 | 5.692568 | 0 |
| AhTE0188 | - | 4.11 |
| AHGS1910 | 19.035441 | 27.49 |
| SNP1 | 28.63 | - |
| ARS729 | 31.451985 | 32.03 |

| | | |
|---|---|---|
| ARS721 | 22.130732 | 34.56 |
| AHGS1465 | 20.917612 | 56.67 |
| AHGS1389 | 37.76887 | 78.21 |
| AHGS3363 | 42.634304 | 78.52 |
| AHGS1646 | 43.349687 | 78.83 |
| SNP2 | 52.87 | - |
| SNP3 | 57.55 | - |
| AHGS4584 | 57.79209 | 80.73 |
| SNP4 | 59.65 | - |
| SNP5 | 59.79 | - |
| SNP6 | 66.38 | - |
| GM672 | 72.196023 | 83.28 |
| SNP7 | 72.67 | - |
| Ah21 | 89.295856 | 84.22 |
| GM1661 | 89.295751 | 82.22 |
| Ah126 | 89.295748 | 84.22 |
| GM1694 | 90.064266 | 85.46 |
| AHGS1713 | - | 90 |
| AHGS1760 | 92.841619 | 92.57 |
| GM2350 | - | 103.02 |
| TC3H02 | - | 130.17 |
| AhTE0571 | 103.985083 | 139.01 |
| seq8E12 | 104.139715 | 145.06 |
| AhTE0499 | 103.336438 | 149.76 |
| AHGS1351 | 105.122604 | 157.85 |

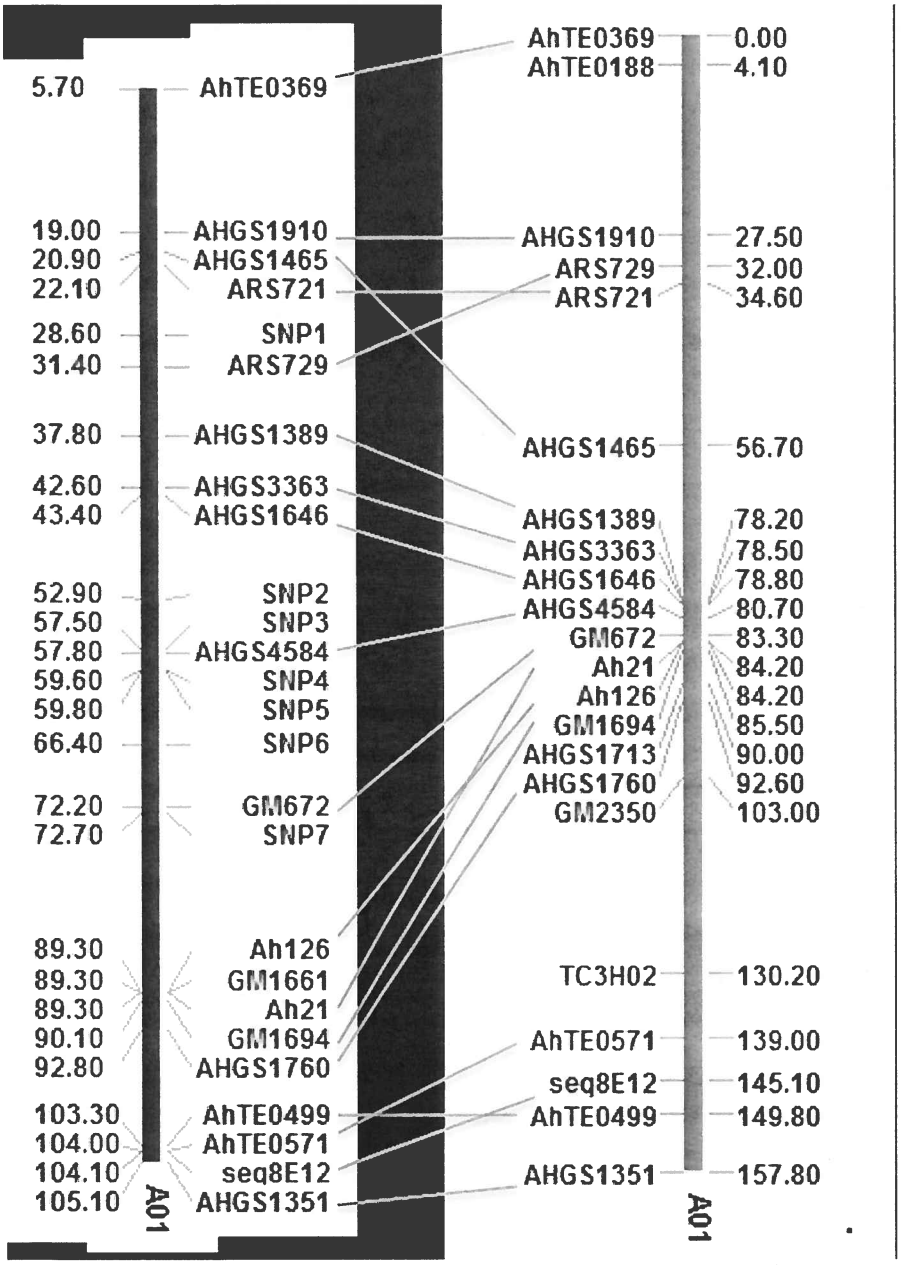


**Figure 2.** Physical and linkage map showing the position of SSR and SNP markers on chromosome A01. Lines indicated the same markers on both maps. a) physical map with the numbers indicating Megabase (Mb); b) linkage map with numbers indicating Centimorgan (cM).

QTL analysis reveal two QTLs were detected on chromosome A01 (Figure 3) with flanking markers, AHGS4584 (80.73 cM) and GM672 (83.28 cM), and AHGS1646 (78.83cM) and AHGS4584 (80.73cM), respectively (Table 2). The two QTLs showed the highest LOD score (9.00 and 3.76 repectively) and high phenotypic variation explained (PVE) (22.7% and 10.02%) respectively) (Table 2). Most likely they are the same QTL with a slight shift to different positions due to the environment effects. In addition, genotype-by-environment interaction, population size, marker density, and genotyping errors can also influence the QTL position. Therefore, most likely one major QTL on chromosome A01 with 23% PVE controls the spotted wilt disease resistance in

Florida-EP™ '113' with two flanking markers AHGS4584 and GM672. Further genotyping using these flanking markers and SNPs will be done on the fine-mapping population above.

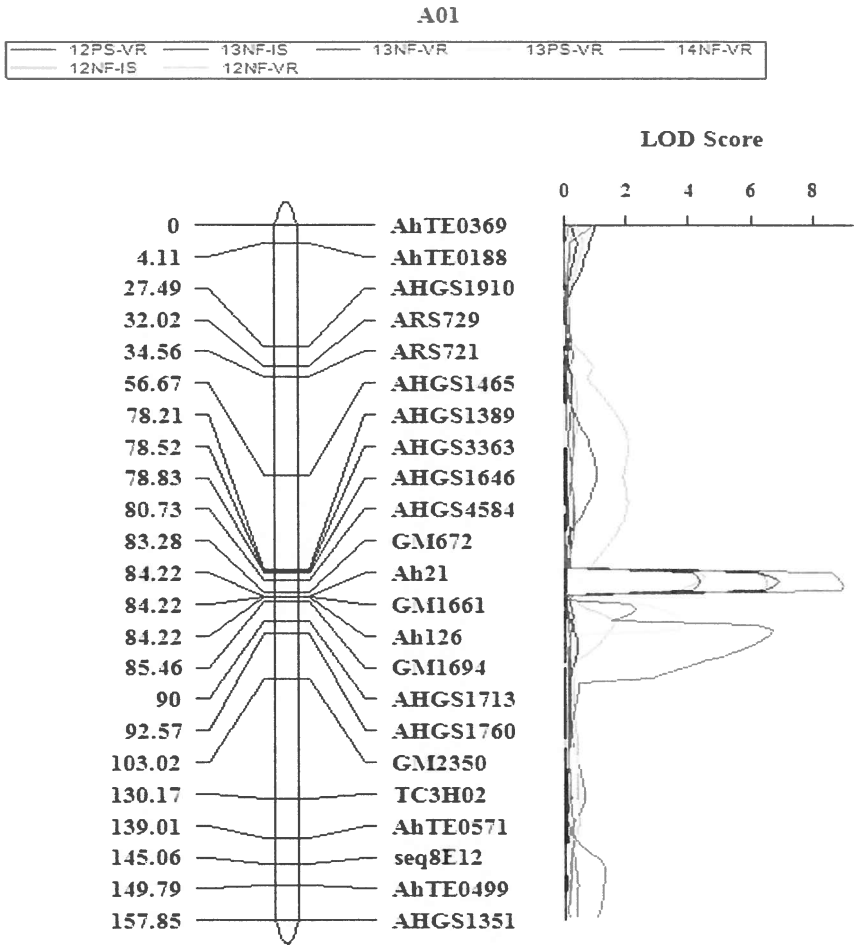


Figure 3. Linkage group with SSR marker positions and the detected QTLs indicated by different color peaks indicating different phenotyping datasets.

Table 2. The positions, flanking markers, LOD values, PVE (%) and additive effects of putative QTLs on A01 chromosome in cross between Florida-EP™ '113' and Georgia Valencia peanut cultivars.

| Position (cM) | Left marker | Right marker | LOD | PVE(%) | add |
|---|---|---|---|---|---|
| 90 | AHGS1713 | AHGS1760 | 3.76 | 10.02 | -0.24 |
| 82 | AHGS4584 | GM672 | 9 | 22.7 | -0.17 |