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2017

Annual Report + Summ

PID 478

I. Realizing molecular marker assisted selection of TSWV resistant lines in the peanut

II. Principle Investigators: Jianping Wang and Barry L. Tillman, Agronomy Department, University of Florida, 3105B McCarty B, Gainesville, FL 32611

III. Project final report in cycle 01-01-18~12-30-18

The **specific research objectives** are to validate the molecular markers closely linked to the major TSWV resistance gene(s) and develop a practical clinical assay for MAS in peanut breeding programs. The results from the project are summarized below:

1. To validate the markers linked to TSWV resistance, populations derived from Florida-EP™ '113' were genotyped using the markers, AHGS3363 and AHGS1646 in the major QTL of TSWV resistance. In one population of 163 individuals, 38 individuals had resistance allele at both loci, and 33 (87%) of the 38 individuals showed TSWV resistance. Therefore, these two markers were considered as validated for marker assisted selection (MAS), which can be used for PCR and polyacrylamide gel electrophoresis (PAGE) for TSWV resistance selection in the lab (Figure 1).

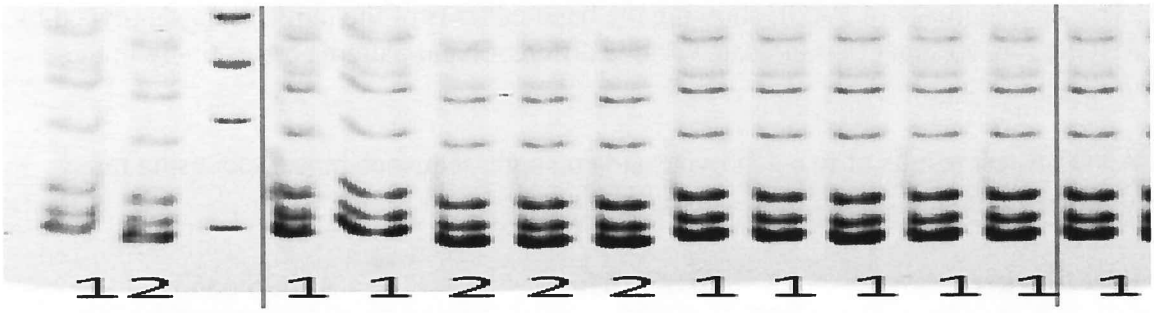

Figure 1. The sample image of PAGE showing the band patterns of the individuals genotyped by marker, AHGS1646. 1 and 2 indicate the band pattern assembled the susceptible and resistance parents, respectively.

2. To survey the prevalence of the resistance allele of the TSWV resistance markers we identified, AHGS3363 and AHGS1646, we genotyped the 107 mini core germplasm collection using the two markers on the PAGE system. Because of the wide genetic diversity of the US peanut mini-core germplasm, the 107 mini-core accessions showed multiple band patterns at both SSR loci with six and eight band patterns at AHGS 3363 and AHGS 1646, respectively (Figure 2). For AHGS 3363, 22 accessions showed the band pattern of resistant Florida-EP™'113'. At the AHGS 1646 locus, 27 accessions showed resistant band pattern. Only one showed the resistance band pattern at both marker loci. Within the US peanut mini-core germplasm, large differences in spotted wilt resistance, including both visual and ELISA ratings, were observed. At marker locus AHGS 1646, the averages of visual ratings in 2012, 2016, and the ELISA of the accession groups showing susceptible and resistant band patterns were 4.14 vs 4.52, 4.15 vs 4.31, and 5.5 vs 5.45, respectively. At marker AHGS 3363, they were 4.44 vs 3.92, 4.49 vs 3.99, and 4.58 vs 5.25, respectively. No statistically significant differences were

observed between the susceptible and resistance genotypes at either of the SSR locus (Table 1). From the results we had so far, we can see that the resistance allelic region we used for the marker development was likely originated from a unique genetic resource, not detectable or represented within the mini core collection. These markers should be used for the TWWV MAS for the plant materials derived from Florida-EP™ '113' or its possible contributing ancestor plant, PI 576638, a *hirsuta* type line originated in the highlands of Mexico and it's relative NC94022 developed in NC at the USA.

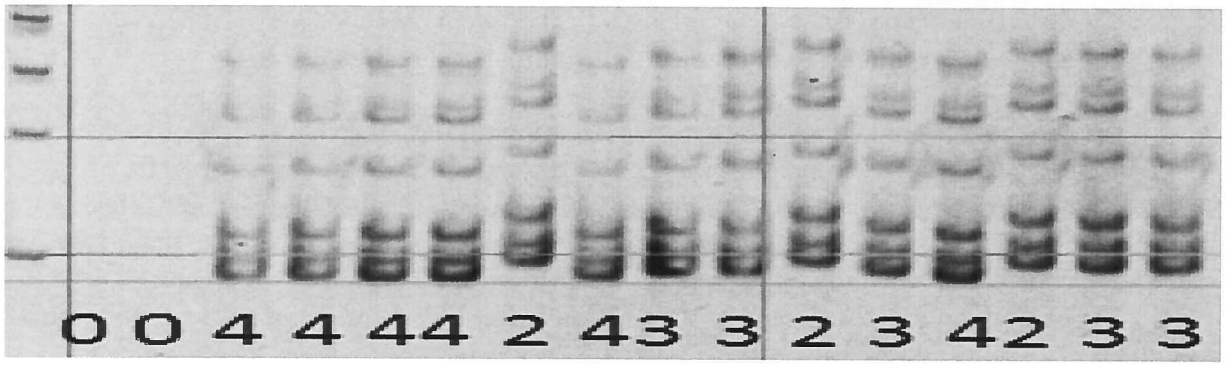

Figure 1. The sample image of PAGE showing the band patterns of the individuals genotyped by marker, AHGS1646. Number 0~4 indicate various different band patterns respectively in the minicore collection.

Table 1. ANOVA test results of two genotypes at two single sequence repeat loci using three different datasets in the US peanut mini-core germplasm

SSR Marker	**AHGS 1646**			**AHGS 3363**		
Genotype(S/R)	S	R	*P*	S	R	*P*
2012 Visual	4.14	4.52	0.54	4.44	3.92	0.13
2016 Visual	4.15	4.31	0.73	4.49	3.99	0.18
ELISA	5.5	5.45	0.94	4.58	5.25	0.48

S band pattern following spotted wilt susceptible cultivar Georgia Valencia, *R* band pattern following spotted wilt resistant cultivar FL-EP™'113'; *P* = *P*-value

3. To develop a rapid and cheap marker diagnose system, we have firstly optimized a simple and rapid DNA extraction process for successful PCR by only homogenize one peanut leaflet in DNA extraction buffer and centrifuge to retrieve the supernatant for PCR. Next we will further fluorescently label the SSR markers for multiplexing in an ABI detection system to eliminate the labor consuming PAGE process. In addition, the SNPs in the marker QTL interval will be tested and allele specific PCR based SNP genotyping method will be developed. These parts will be accomplished in the next cycle of the project.