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Final Report of APPA-RIA16-PID 456

I. Abstract. Cultivated peanut (*Arachis hypogaea L.*) is an important oilseed crop that is grown extensively in Africa, Asia and America. The diseases early and late leaf spot severely constrains peanut production worldwide. Because multiple genes control resistance to leaf spot diseases, conventional breeding is a time-consuming approach for pyramiding resistance genes into a single genotype. Marker-assisted selection (MAS) would complement and accelerate conventional breeding once molecular markers tightly associated with the resistance genes are identified. In this study, we have generated a large number of SNPs through genotyping by sequencing (GBS) and constructed a high-resolution map with an average distance of 1.34 cM among 2,753 SNP markers distributed on 20 linkage groups. QTL mapping has revealed that major QTL within a confidence interval could provide an efficient way to detect putative resistance genes. Analysis of the interval sequences has indicated that a major QTL for resistance to late leaf spot anchored by two NBS-LRR resistance genes on chromosome B05. Two major QTLs located on chromosomes A03 and B04 were associated with resistance genes for early leaf spot. Sequences within the confidence interval would facilitate identifying resistance genes and applying marker-assisted selection for resistance.

Project Title: Identification of leaf spot resistant genes in peanuts utilizing high-throughput GBS sequencing technology and disease association

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Cooperating Personnel: None.

Summary: Cultivated peanut (*Arachis hypogaea L.*) is an important oilseed crop that is grown extensively in Africa, Asia and America. The diseases early and late leaf spot severely constrains peanut production worldwide. Because multiple genes control resistance to leaf spot diseases, conventional breeding is a time-consuming approach for pyramiding resistance genes into a single genotype. Marker-assisted selection (MAS) would complement and accelerate conventional breeding once molecular markers tightly associated with the resistance genes are identified. In this study, we have generated a large number of SNPs through genotyping by sequencing (GBS). Analysis of the interval sequences has indicated that a major QTL for resistance to late leaf spot anchored by two NBS-LRR resistance genes on chromosome B05. Two major QTLs located on chromosomes A03 and B04 were associated with resistance genes

for early leaf spot. Sequences within the confidence interval would facilitate identifying resistance genes and applying marker-assisted selection for resistance.

II. Main Body of Report

Project Title: Identification of leaf spot resistant genes in peanuts utilizing high-throughput RNA sequencing technology and disease association

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1) Objectives: Identify the genes that correspond resistance to early leaf spot, caused by *Cercospora arachidicola*, and late leaf spot, caused by *Cercosporidium personatum*, using our identified lines with high resistance to leaf spot from previously funded project as candidate genotypes;
2) Create new germplasm by pyramiding multiple genes in a single plant for enhancing leaf spot resistance in peanut cultivar improvement.

Procedures: Selected lines were challenged with leaf spot pathogens and infected leaf samples will be utilized as experimental samples. These genotypes will be planted in randomized complete block (RCB) design with three replicates in Headland, AL in 2016. All plant will be infected in none fungicide-treated natural field-based infection. Leaf tissue of each genotype will be collected for genotyping by sequencing (GBS). Data will be analyzed using bioinformatic tools to identify the genes related with leaf spot resistance.

Results and Discussion:

QTL analysis of genotyping data with phenotyping data in 184 RIL lines was carried out for ELS and LLS disease resistances using WinQTLCart version 2.5. As a result, 15 genomic regions

were identified associated with resistance to leaf spot diseases in the Florida-07 x SPT06-06 population within three years. For LLS, eight QTLs were identified in six linkage groups between two years, among which 2 major QTLs (qLLS2015-B05-2 and qLLS2016-B05) were mapped the same position in the B05 group with 11.64 and 16.6% PVE in two years, respectively (Table 2). Although another QTL (qLLS2015-B05-1) was mapped on the opposite end of the same linkage group, its physical position was nearby the region of these two major QTLs (Figure 2). Two major QTLs for ELS were located on different linkage groups, A03 and B04 accounted for 11.67 and 10.63% PVE in 2016 and 2017, respectively. The remaining QTLs exhibited <10% PVE. To validate these identified QTLs in this study, three major QTLs with PVE >10% (qLLS2016-B05, qELS2017-A03, and qELS2016- B04) were selected to investigate the sequences within their intervals. QTL mapping on this high-density map generated narrow QTL intervals with sequence length ranging from 1.5 Mbp (qELS2017-A03), 2.4 Mbp qLLS2016-B05), to 12.5 Mbp (qELS2016-B04), which facilitates us to scan intervals for resistance gene content. Although these three intervals were still quite wide, they could be downloaded from reference genomes (https://peanutbase.org) based on their physical locations.

Discoveries: Describe any Discoveries that were made in the course of the project. Explain whether any Discovery is potentially patentable or protectable under Title 35 of the U.S. Code or the Plant Variety Protection Act. In addition, please describe any public disclosures that have been made related to the Discovery, including conference presentations or papers.

Analysis and annotation of genes within confidence intervals of major QTLs, two NBS-LRR resistance genes for LLS and two homologs of TMV resistance protein N for ELS were revealed. The identified genomic regions and putative resistance genes to early and late leaf spots will be further studied for application of the MAS approach in peanut breeding.