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I. Genetic mapping the gene loci underlying white mold disease resistance in peanut

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III. Project report for funding period 03-05-2013~12-31-2013

Phenotyping the white mold disease of the segregating population: The recombinant inbred line (RIL) population derived from cross between NC3303, the white mold resistant, and Tifrunner, the white mold susceptible cultivars were provided by Dr. Correy Holbrook at Tifton Gerogia in May, 2013. Totally, we received 146 RILs from Dr. Hulbrook. Due to the seeds number limitation of some RILs, 54 RILs had enough seeds and were planted in three replicates, 13 RILs had enough seeds to be planted in two replicates and 79 RILs were in one replicate in Citra Florida, May 2013. When the plant canopies of the two rows in the plot were reached to each other (Figure 1A), the white mold inoculum (Figure 1D) was applied. For the inoculum preparation, the sclerotium from white mold were collected from three different areas (105, 148, 197) in Florida, which were then grown and inoculated on the corn and wheat in flask for fungus increase (Figure 1D). For inoculation, the inoculum was diluted using fresh carked corn and fresh wheat. Two inoculation methods were performed: flagged point inoculation method (Figure1B, 1F) in one row of the plot and spray inoculation (Figure 1C) on the other row of the plot on July 24th in the field.

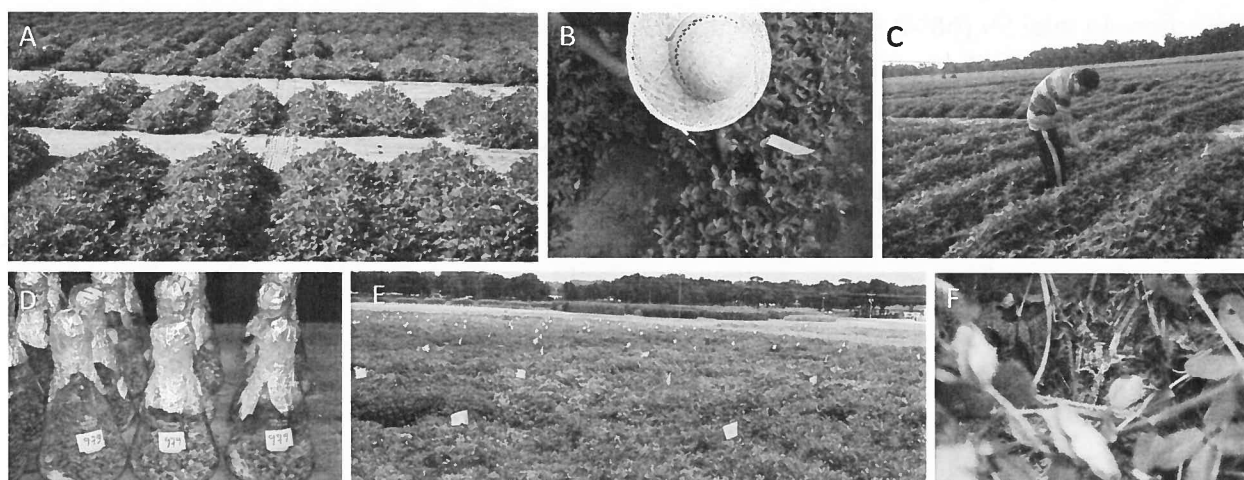


Figure 1. White mold disease inoculation in the field. A. field plot display before inoculation. B. Point inoculation process; C. Plot spreading inoculation; D. Inoculum preparation; E. field plot display after inoculation; F. white mold disease development 8 days after inoculation.

The fungal growth and propagation were checked weekly after inoculation. For disease measurement, both disease incidence and percent severity were recorded within each plot. Percent of infection were observed in the plant closest to the inoculation point. 0%= no symptoms and 100% plant is wilting, with necrosis and dead (Figure 2).

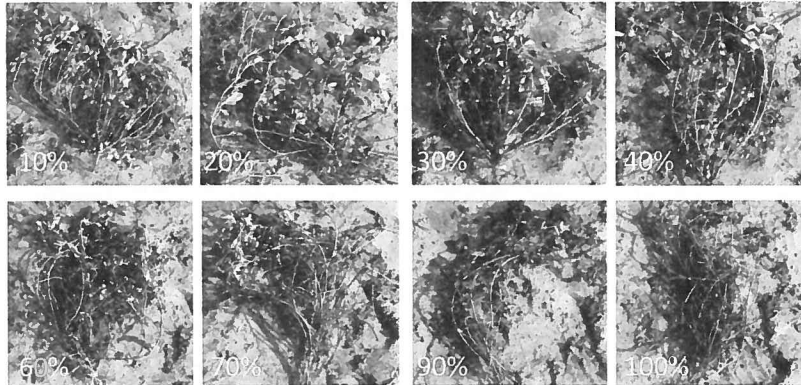


Figure 2. The example of white mold severity score scales.

The white mold disease incidence distributed from 0 to 2 (Figure 3A) with 68% of RILs showed certain level of incidence and 32% of RILs had no incidence, which could be either escaped from inoculation or showed strong resistance. Of the 68% RILs showing disease incidence, majority of lines showed an incidence of 1-1.5 with generally a normal distribution (Figure 3A). The disease severity recorded three weeks after inoculation showed that majority of the RILs had less than 50% disease severity (Figure 3B). A second severity score was performed after the plots were pulled to see if there was more fungal growth at the soil level. Results from the second scoring showing a much wider distribution though still more lines showed less than 50% disease severity (Figure 3C) indicating further disease development after three weeks of infection. In total 99 (68%) RILs showed 30% or higher disease severity. The correlation coefficient between the incidence and infection severity recorded three weeks after infection is 0.65 (Figure 4A) with a significant positive correlation. The correlation between severity rate scored on the three weeks after infection and after digging (Figure 4B) is also significant with a correlation coefficient of 0.44. These phenotypic results indicated that this RIL population derived from the cross between NC3303, the white mold resistant, and Tifrunner, the white mold susceptible cultivars is segregating for the white mold disease resistance.

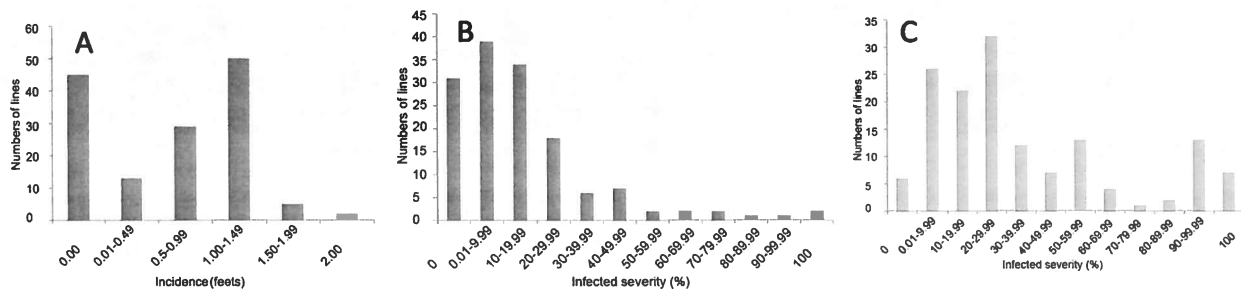


Figure 3. The white mold disease resistance performance of the segregating population. A. the disease incidence distribution. B. the disease severity (%) distribution scored three weeks after inoculation. C. the disease severity (%) distribution scored after digging in the field.

Flowering time, yield, and seed coat color of the segregating population: The flowering of each plot was recorded on 35 days after planting. In total, 64 RILs had not flowered by 35 days after planting, and the rest of lines bloomed flowers on 2% - 70% plants in each plot (Figure 4A). The 146 RIL plants were harvested on September 26th 2013, threshed and graded for yield. The plot yield showed a wide normal distribution ranging from 0 to 10 pounds per plot with most of the lines showing a yield of 6-7 pounds per plot (Figure 4B). The seed coat color ranged from yellowish tan to dark red, with most of the lines showing tan seed coat color (Figure 4C). The results further indicated that this RIL population was segregating on flowering time, yield trait, and seed coat color as well in addition to the white mold disease resistance due to the significant variance between the two parent lines.

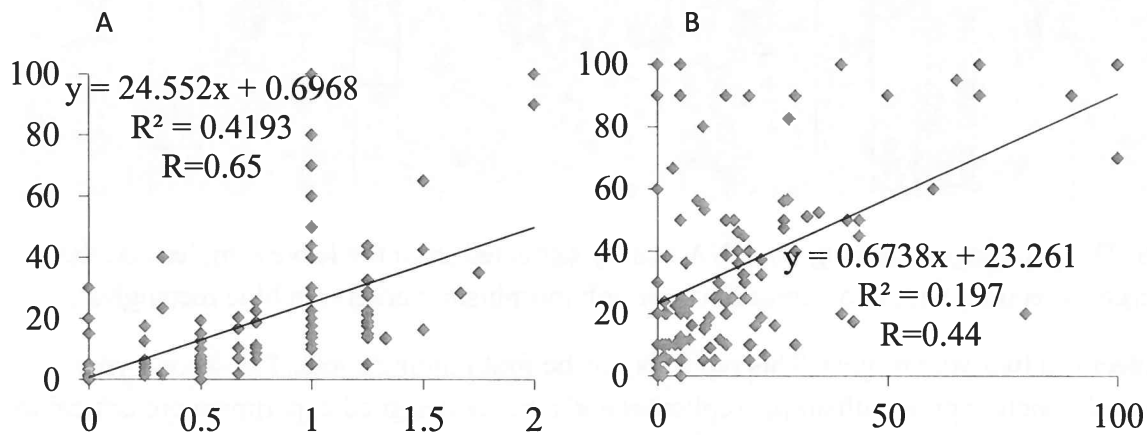


Figure 4. The correlation between the white mold disease incidence and the severity rate (%) scored on three weeks after infection (A) and between the two severity rates recorded on three weeks after infection and after digging (B).

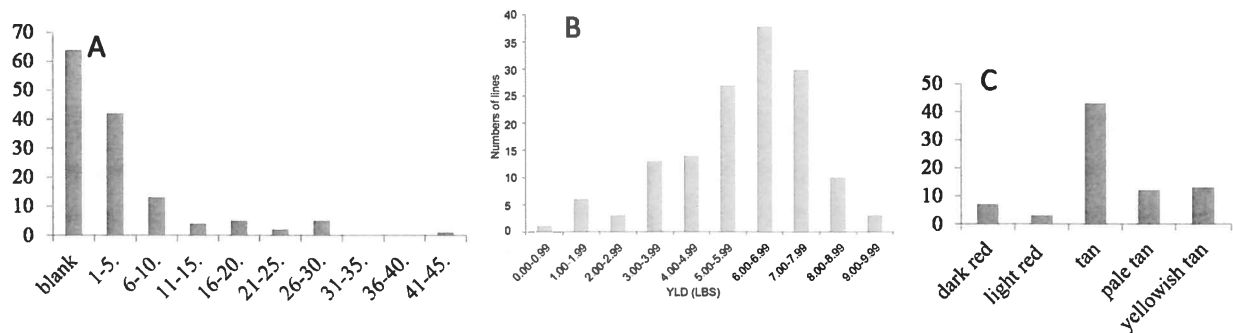


Figure 5. The flowering, yield, and seed coat color distribution of the segregating population. A. the number of RILs with flowers on 35 days after planting. B. the yield distribution of the population after harvest. C. the seed coat color distribution.

Genotyping the segregating population: Two sets of unopened leaves samples were collected for all 146 entries and the parents for DNA extraction (Figure 6A). The DNA samples of the two parental lines were used to screen the polymorphic Simple Sequence Repeat (SSR) markers. In total, 756 SSR markers distributed randomly on 20 linkage groups were screened and 56 polymorphic markers were identified (Figure 6B).

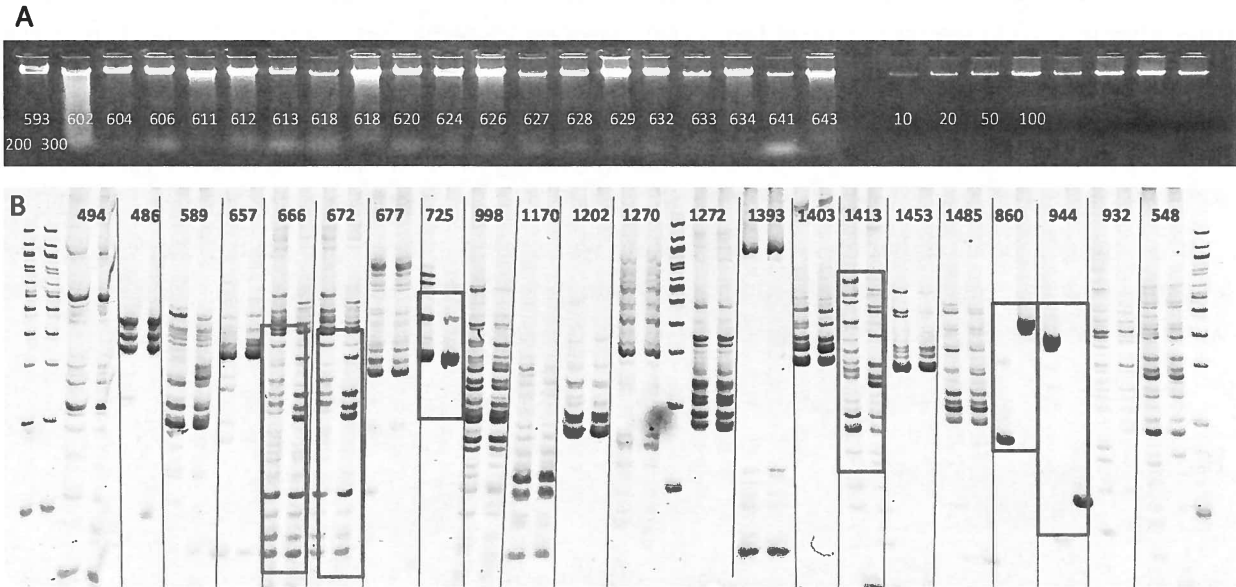


Figure 6. The gel images showing the DNA quality extracted from the leave samples and the SSR marker screening with two parent lines for polymorphism markers (in blue rectangles).

This project is a two-year project. This report is for the first funding cycle. The second year repeated field phenotyping with ample replicates and a better designed experiment are critical to study the white mold disease segregation in this population, which is the base for the resistance gene mapping. For the genotyping, we are expecting to use the screened polymorphic SSR markers to genotype the whole segregating population in the second year. A genotyping by sequencing (GBS) approach will also be used to have a wide coverage of the peanut genomes.