Final Report

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PROGRESS REPORT
TO
NORTH CAROLINA PEANUT GROWERS ASSOCIATION, INC.

TITLE: **Marker Assisted Selection For Sclerotinia Blight Resistance in Virginia-Type Peanuts.**

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DEPARTMENT: **Crop Science**

PROGRESS:

Sclerotinia blight is one of the most important yield- and quality-limiting diseases of peanut in the Virginia-Carolina region. The disease, caused by the soil-borne pathogen Sclerotinia minor, can cause substantial losses either in the form of lost yield and quality or as a cost of control. Development of resistant or tolerant cultivars is one of the most important components of the array of cultural practices designed to manage this disease. Moreover, chemical control is costly and any savings that could be realized by deployment of resistant cultivars would apply directly to the growers' margin of profit.

Measurement of Sclerotinia resistance is not simple. Because the pathogen is soil-borne, its distribution in the soil can be patchy. Moreover, expression of Sclerotinia resistance in the field is highly affected by environmental conditions. These factors confound the separation of genetic from non-genetic factors in disease reaction and difficult selection of resistant materials. Marker assisted selection (MAS) is a technique used to improve the efficiency of selection by using DNA technology to determine the presence of desirable genes rather than waiting for gene expression. When the markers co-segregate with the trait of interest MAS can be used to transfer (a) specific gene(s) into a desired genetic background. Because molecular markers generally are not influenced by the environment, superior genotypes can be identified with fewer tests.

In the summer of 2005, crosses were made between Sclerotinia-resistant line N96074L, a sister line of germplasm release N96076L that actually has a slightly better Sclerotinia reaction, with the widely grown tolerant virginia-type cultivar Perry. F_1 hybrids from this cross were sent to the 2005-2006 Puerto Rico Winter Nursery (PRWN) to obtain an F_2 segregating population. In order to develop recombinant inbred lines (RILs) needed to associate markers with resistance, the F_2, F_3, F_4, and F_5 generations were advanced by single-seed descent, two generations per year, one in North Carolina and one at the PRWN. Given that assessment of resistance in both field testing and artificial inoculations is difficult because of the effect of the environment on disease response, testing of lines versus individuals will be advantageous in obtaining more accurate estimates of disease response. $F_{6:7}$ families were harvested in bulk at the 2008-2009 PRWN, the seed shelled, and a replicated field test of 110 families along with the two parents and nine resistant and susceptible checks was conducted at an infested site in Bertie County. Conditions of development of Sclerotinia blight were excellent, and there was significant variation among the RILs (Table 1).

For MAS to be useful, it is necessary to identify large numbers of DNA markers that are polymorphic, *i.e.*, that assume measurably different forms, between the parents of the population. Then the polymorphic markers are assessed in the RILs and statistical procedures are used to determine the degree of association between the markers and the expression of

Sclerotinia resistance. MS student Franco Villegas is currently assessing the two parents for their genotypes at several hundred markers known to be variable among some peanuts but not necessarily between Perry and N96074L. This work is being conducted in the University of Georgia lab formerly led by Dr. Steve Knapp. Dr. Knapp has graciously allowed Franco to search through the hundreds of markers available in the Knapp lab, freeing us from the need of buying the expensive DNA supplies necessary for the task. We will buy the supplies ("primers" used in the polymerase chain reaction that makes the markers readable) for the markers that Franco finds to be polymorphic in the Perry / N96074L population. In other crosses, we have identified 150 to 200 polymorphic markers; we hope to find a similar number for Perry / N96074L. Such a number should provide sufficient coverage of the peanut genome. We will evaluate recombinant inbred lines along with the two parents in the greenhouse using the artificial inoculation method developed by Hollowell et al. (2008). Lines will be analyzed with the 200 polymorphic markers to generate a linkage map in order to screen the genome for genetic factors controlling resistance to the fungus. Perry is somewhat tolerant to the fungus, but the sources of resistance are different in both parents. Resistance in N96074L is most likely derived from the diploid wild species *Arachis cardenasii* that is a distant ancestor. Therefore, we should be able to identify genetic factors contributing to Sclerotinia resistance in both parents. Markers found to be associated with resistance can be used to aid in transferring the resistance present in N97074L into elite peanut breeding materials. Using MAS, superior genotypes can be identified more rapidly and with more precision than by relying solely on disease pressure in the field. The populations will be advanced through conventional variety development while being evaluated for presence of markers as means to selecting for Sclerotinia resistance.

Table 1. Adjusted mean Sclerotinia incidence among 110 recombinant inbred lines developed from a cross of Perry / N96074L, the parents, and susceptible and resistant checks.

Group / Line	Proportion of symptomatic plants		Square root transformation	
	Mean±SE	Rank	Mean±SE	Rank
Perry / N96074L	**0.394±0.011$^{\alpha}$**		**0.599±0.010$^{\beta}$**	
Perry / N96074L (F2-S-S-S-S-002: F08)	0.464±0.116	80	0.681±0.109^{z}	87
Perry / N96074L (F2-S-S-S-S-004: F08)	0.891±0.116^{z}	121	0.943±0.109^{z}	121
Perry / N96074L (F2-S-S-S-S-005: F08)	0.339±0.116^{a**}	48	0.572±0.109	48
Perry / N96074L (F2-S-S-S-S-006: F08)	0.339±0.116^{a**}	48	0.555±0.109	44
Perry / N96074L (F2-S-S-S-S-007: F08)	0.396±0.116	63	0.624±0.109	66
Perry / N96074L (F2-S-S-S-S-008: F08)	0.192±0.116^{a†}	10	0.436±0.109^{a**}	13
Perry / N96074L (F2-S-S-S-S-009: F08)	0.339±0.116^{a**}	48	0.542±0.109	41
Perry / N96074L (F2-S-S-S-S-010: F08)	0.558±0.116	103	0.746±0.109^{z}	104
Perry / N96074L (F2-S-S-S-S-011: F08)	0.625±0.116^{z}	113	0.787±0.109^{z}	114
Perry / N96074L (F2-S-S-S-S-012: F08)	0.063±0.116^{a†}	2	0.177±0.109^{a†}	2
Perry / N96074L (F2-S-S-S-S-013: F08)	0.111±0.116^{a†}	4	0.334±0.109^{a†}	5
Perry / N96074L (F2-S-S-S-S-014: F08)	0.423±0.116	70	0.616±0.109	62
Perry / N96074L (F2-S-S-S-S-015: F08)	0.467±0.116	81	0.634±0.109	70
Perry / N96074L (F2-S-S-S-S-016: F08)	0.241±0.116^{a*}	16	0.476±0.109**	18
Perry / N96074L (F2-S-S-S-S-017: F08)	0.727±0.116^{z}	120	0.850±0.109^{z}	120
Perry / N96074L (F2-S-S-S-S-018: F08)	0.500±0.116	92	0.704±0.109^{z}	92
Perry / N96074L (F2-S-S-S-S-019: F08)	0.654±0.116^{z}	116	0.800±0.109^{z}	115
Perry / N96074L (F2-S-S-S-S-020: F08)	0.579±0.116^{z}	107	0.760±0.109^{z}	108
Perry / N96074L (F2-S-S-S-S-021: F08)	0.393±0.116	62	0.626±0.109	67
Perry / N96074L (F2-S-S-S-S-022: F08)	0.554±0.116	100	0.743±0.109^{z}	100
Perry / N96074L (F2-S-S-S-S-023: F08)	0.373±0.116^{a}	54	0.595±0.109	57
Perry / N96074L (F2-S-S-S-S-024: F08)	0.387±0.116	58	0.594±0.109	56
Perry / N96074L (F2-S-S-S-S-025: F08)	0.284±0.116^{a**}	30	0.503±0.109	28
Perry / N96074L (F2-S-S-S-S-026: F08)	0.600±0.116^{z}	111	0.772±0.109^{z}	111
Perry / N96074L (F2-S-S-S-S-027: F08)	0.494±0.116	90	0.684±0.109^{z}	88
Perry / N96074L (F2-S-S-S-S-028: F08)	0.339±0.116^{a**}	48	0.581±0.109	50
Perry / N96074L (F2-S-S-S-S-029: F08)	0.554±0.116	100	0.743±0.109^{z}	100
Perry / N96074L (F2-S-S-S-S-030: F08)	0.512±0.116	93	0.712±0.109^{z}	93
Perry / N96074L (F2-S-S-S-S-031: F08)	0.577±0.116^{z}	106	0.760±0.109^{z}	107
Perry / N96074L (F2-S-S-S-S-032: F08)	0.238±0.116^{a*}	15	0.478±0.109**	19
Perry / N96074L (F2-S-S-S-S-033: F08)	0.324±0.116^{a**}	40	0.569±0.109	47
Perry / N96074L (F2-S-S-S-S-034: F08)	0.518±0.116	95	0.720±0.109^{z}	95
Perry / N96074L (F2-S-S-S-S-035: F08)	0.268±0.116^{a*}	25	0.448±0.109^{a**}	15
Perry / N96074L (F2-S-S-S-S-036: F08)	0.262±0.116^{a*}	23	0.490±0.109	24
Perry / N96074L (F2-S-S-S-S-037: F08)	0.339±0.116^{a**}	48	0.542±0.109	41
Perry / N96074L (F2-S-S-S-S-038: F08)	0.482±0.116	87	0.691±0.109^{z}	90
Perry / N96074L (F2-S-S-S-S-039: F08)	0.408±0.116	66	0.639±0.109^{z}	73
Perry / N96074L (F2-S-S-S-S-040: F08)	0.554±0.116	100	0.743±0.109^{z}	100
Perry / N96074L (F2-S-S-S-S-041: F08)	0.280±0.116^{a**}	28	0.512±0.109	32
Perry / N96074L (F2-S-S-S-S-042: F08)	0.664±0.116^{z}	117	0.811±0.109^{z}	117
Perry / N96074L (F2-S-S-S-S-043: F08)	0.271±0.116^{a**}	27	0.507±0.109	31
Perry / N96074L (F2-S-S-S-S-044: F08)	0.255±0.116^{a*}	20	0.501±0.109	27
Perry / N96074L (F2-S-S-S-S-045: F08)	0.271±0.116^{a**}	26	0.520±0.109	35
Perry / N96074L (F2-S-S-S-S-046: F08)	0.259±0.116^{a*}	21	0.495±0.109	25
Perry / N96074L (F2-S-S-S-S-047: F08)	0.233±0.116^{a*}	14	0.450±0.109^{a**}	16
Perry / N96074L (F2-S-S-S-S-048: F08)	0.252±0.116^{a*}	19	0.442±0.109^{a**}	14
Perry / N96074L (F2-S-S-S-S-049: F08)	0.409±0.116	67	0.638±0.109^{z}	72
Perry / N96074L (F2-S-S-S-S-050: F08)	0.333±0.116^{a**}	43	0.558±0.109	46
Perry / N96074L (F2-S-S-S-S-052: F08)	0.446±0.116	76	0.667±0.109^{z}	81
Perry / N96074L (F2-S-S-S-S-053: F08)	0.331±0.116^{a**}	41	0.518±0.109	34

(cont'd)

α,β,γ Group means followed by the same Greek letter are not different by t-test (P<0.05).
a,b,c Line means followed by the same Roman letter are not different by t-test (P<0.05).