i. **INCREASING RESISTANCE TO ROOT-KNOT NEMATODE IN COMMERCIAL PEANUTS FOR THE SE**

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Materials and Methods

SCAR Analysis

Forward primer, SCZ3-FO1 5'-CAGCACCGCAGCATAAAAAC-3' and reverse primer, SCZ3-RO2 5'-CAGCACCGCACACATTCTGG-3' were used to amplify a SCAR marker with an expected size of 310 bp. PCR reactions were performed with a MJ Research thermocycler (Waltham, MA) under the following conditions: 94 °C for 5 min, 30 cycles at 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 3 min. The root-knot nematode susceptible cultivar, Florunner, was used as a negative control and the root-knot nematode resistant cultivar, NemaTAM was used as a positive control for all experiments.

Oleic Acid Analysis

Seeds from fifty F_2 plants that were positive for the SCAR marker and agronomically superior were then analyzed by George D. Person (laboratory technician, University of Florida) using fourier transform infrared (FTIR) spectrometer (Nexus 870 FTIR, Thermo Nicolet, Madison, WI) for oleic acid content. Seeds were then characterized as high oleic (> 75% oleic acid content), medium oleic (60 – 75% oleic acid), and low oleic (< 60% oleic acid). These seeds were then planted at the University of Florida Pine Acres Research Station, Citra, FL. Leaf tissue

was collected from 50 of the resulting plants. The tissue was quick frozen in liquid nitrogen and stored at –80 °C until use for RFLP analysis.

RFLP Analysis

Peanut genomic DNA (20 µg) was digested overnight with EcoRI according to the manufacturer's instructions (New England Biolabs, Beverly, MA). Digested DNA was subjected to electrophoresis for 16 h using 0.8% agarose gels and transferred onto Hybond N+ membranes (Amersham, Arlington Heigts, IL) by capillary blotting (Southern, 1975) and UV cross linked for 3 min at 70,000 micro-joules/cm^2. The RFLP probe, R2430E, was kindly provided by Dr. Gregory T. Church (Texas A&M University). Probe DNA (50 ng) was labeled with α-P^{32} dCTP by random primer extension (Feinberg and Vogeltein, 1983). Pre-hybridization and hybridization were performed at 65 °C with 7% SDS and denatured salmon sperm DNA (Church and Gilbert, 1984). Samples were washed three times for 20 min each at 65 °C with 0.5X SSC and 0.1% SDS. Hybridized blots were autoradiographed using X-ray film (Kodak XAR-5) and two intensifying screens at –70 °C for three to 10 d.

Results

The root-knot nematode resistant and susceptible alleles detected using R2430E were distinct and easy to score for lines F1334, F79x4-6, F94x30-8-2-1-b3, F94x30-8-3-1-b2, F94x30-8-2-2-b3 and F94x30-5-2-3-3-b3. All lines with a susceptible RFLP genotype had a susceptible phenotype based on root-knot nematode reproduction . Field results showed that the gall and egg mass index for the root-knot nematode resistant cultivar COAN was 1, whereas the mean gall and egg mass index on roots and pods of the susceptible cultivar Florunner was 3.2 . Reproduction of *M. arenaria* on each of the lines as measured by the number of galls and egg masses on roots ranged from 3.0 to 3.9 with greater than 11% egg masses on pods and pegs

which was more than the reproduction found on Florunner ($P < 0.001$) . No resistance to *M. arenaria* was observed in any of the six Florida breeding lines.

Consequently, to incorporate root-knot nematode resistance into the Florida germplasm along with the high oleic trait, several crosses involving the root-knot nematode resistant cultivar COAN and susceptible high oleic cultivars HULL (Gorbet, 2007), ANorden (Gorbet, 2007), UF 98326 and lines F89xOL14-1-4-1-1-1-2, and 92x0L19-8-1-1 were made and the seed from F_1 plants was screened for root-knot nematode resistance with the SCAR marker Z3/265. This marker was chosen over the RFLP marker due to its ease for screening.

A total of 740 seed from F_1 plants were screened for Z3/265 and 193 were positive for the presence of the marker. All the seeds from F_1 plants positive for the presence of Z3/265 were subsequently advanced to the F_2 generation. Leaves from 50 plants that were agronomically sound and disease free were collected and subjected to oleic acid and RFLP analysis with the main objectives of determining a relationship between the presence of the SCAR marker and RFLP marker as a means to test for reliability and zygosity, and for further advancing to the F_3 generation. Thirty of the 50 plants were high oleic (> 75%), 9 were medium oleic (65 - 70%) and 11 (< 60%) were low oleic (Table 4-5). There was no relationship between root-knot nematode resistance and the high oleic acid trait .

Using R2430E, individuals plants were scored as homozygous for resistance (RR) if only the band associated with resistance was present; heterozygous for resistance (RS) if both the band for resistance and susceptibility were present, and susceptible (SS) if the band associated with susceptibility was present. Five (10%) out of 50 F_2 selections were homozygous for the susceptible marker and six (12%) were homozygous for the resistance marker, with the

remainder (74%) being heterozygous. Three of these selections were susceptible and the rest were resistant.

Finally, these 50 F_2 selections were planted in a field highly infested with root-knot nematodes (*M. arenaria* race 1). Resistance to *M. arenaria* race 1 was measured based on number of galls present on peanut roots and pegs. Forty-four selections (88%) were found to be resistant and three selections (18%) were susceptible . For the resistant control NemaTAM, the mean peg and pod gall index was 1 and root gall index was 1, whereas for the susceptible control Florunner, the mean peg and pod gall index was 1 and root gall index was 3. Reproduction of *M. arenaria* on each of the selections as measured by the peg and pod gall index and root gall index ranged from 0.0 to 1 and 1 to 3 respectively. Reproduction of *M. arenaria* on each of the selections as measured by the number of galls on roots, pegs and pods ranged from 0 to greater than 100.

Conclusions

The SCAR marker Z3/265, and the RFLP R2430E analyses of filial generations (F_1 and F_2) of crosses involving the root-knot nematode resistant cultivar COAN and the susceptible cultivars HULL (Gorbet, 2007), and ANorden (Gorbet, 2007) and lines F89xOL14-1-4-1-1-1-2 and 92x0L19-8-1-1, indicated co-segregation of these markers. Based on this, it can be hypothesized that both the markers might be linked to the same root-knot nematode resistance gene.

Resistance in some selections, despite the absence of the R2430E marker was possible because R2430E is 4.2 cM away from the resistance locus, and this indicates a possible recombination of 4% between marker and root-knot nematode resistance . RFLP data were somewhat more reliable than SCAR marker data as there was strong relationship (90% of F_2

selections that were RFLP marker positive were root-knot nematode *M. arenaria* resistant) between the presence of the RFLP marker and root-knot nematode resistance as measured in the field . Moreover, the RFLP marker allowed for the selection of homozygous individuals for root-knot nematode resistance . However, using the RFLP marker resulted in a higher frequency of lost data due to several factors such as insufficient DNA availability, incomplete digestion of DNA, and poor hybridization. In addition, RFLP analysis is more cumbersome and costly than SCAR analysis. SCAR marker analysis is simple, rapid and cost effective when it comes to screening large numbers of breeding lines. The primary drawback of SCAR markers is that they are dominant and do not permit the scoring of heterozygous individuals. However, the SCAR marker Z3/265 derived form *A. cardenasii* is linked at 10 ± 2.5 cM and 14 ± 2.9 cM from the root-knot nematode resistance genes *Mag* and *Mae,* respectively, which indicates a possible recombination frequency of 10 to 14%. This high rate of recombination might result in false positives. Therefore, a more tightly linked SCAR marker would be desirable. Also mature peanut leaf has high amounts of phenolic compounds and polysaccharides; these compounds interfere with *Taq* polymerase affecting the reproducibility of Z3/265 marker. So in order to overcome this problem peanut seed DNA was used in our study to perform SCAR marker analysis.

According to the root gall index of the F_3 generation , there is a discreet reaction with respect to root-knot nematode resistance, but the peg and pod gall index is not as uniform. This can be explained by the recent report that the resistance gene in COAN has two major effects when compared to susceptible cultivars, penetration and further development after penetration.

Marker-assisted selection helps in identifying those individuals in which desirable recombination events have occurred during backcrossing of the nematode resistance gene into elite peanut cultivars. In addition, marker-assisted selection has the potential to significantly

reduce the cost associated with field planting, maintenance and field trails. Furthermore, in this study marker assisted selection allowed for peanut genotypes to be screened without relying on inoculation tests in advanced generations, which in turn reduces the time for developing root-knot nematode resistant peanut cultivars.