Field Test Results Versus Marker Assisted Selection for Root-Knot Nematode Resistance in Peanut

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ABSTRACT

A common set of 12 advanced Georgia peanut (*Arachis hypogaea* L. subsp. *hypogaea* var. *hypogaea*) breeding lines that were derived from 'COAN' cross combinations were compared with three check cultivars for root-knot nematode (RKN) [*Meloidogyne arenaria* (Neal) Chitwood race 1] resistance. These 15 genotypes were grown in RKN populated field tests using a randomized complete block design with three replications for two years (2011 and 2012). Two molecular markers (SCAR 197/909 and SSR-GM565) used for marker assisted selection (MAS) did not agree with low gall ratings and high pod yield for four out of the 15 genotypes (26.7%). The results were the same each year with the same four field RKN-resistant genotypes being incorrectly identified as susceptible (false negatives) by both markers. Reciprocal cross combinations involving field resistant parents showed one-gene difference between MAS resistant × MAS susceptible in F_1 and F_2 populations. The lack of accuracy differentiating resistant RKN breeding lines when using these two markers was attributed to either recombination between the resistant gene *RMA* and these two markers, or the possible identification of a second unlinked nematode resistant gene. Regardless, more tightly-linked molecular markers are needed for RKN-resistance in future MAS breeding programs.

Key Words: *Arachis hypogaea* L., groundnut, pod yield, *Meloidogyne arenaria* (Neal) Chitwood race 1, gall rating, genetic ratio.

Root-knot nematodes (RKN) [*Meloidogyne arenaria* (Neal) Chitwood race 1] are major problems in US peanut (*Arachis hypogaea* L.) production (Kokalis-Burelle and Rodriquez-Kabana, 1997). Consequently, new and improved RKN-resistant cultivars are needed to minimize damage, reduce chemical control costs, and increase yield, grade, and dollar value returns.

'COAN' (Simpson and Starr, 2001) was the first peanut cultivar developed with very high RKN-resistance. The RKN-resistance was introgressed into the cultigen as a single dominant gene, *Rma*, from a wild species cross [*A. batizocoi* Krapov. & W. C. Gregory × (*A. cardenasii* Krapov. & W. C. Gregory × *A. diogoi* Hoehne, formerly *A. chacoense* Krapov. & W. C. Gregory)] (Simpson *et al.*, 1993). 'NemaTAM' (Simpson *et al.*, 2003) was the second peanut cultivar released with further backcrosses to the recurrent parent 'Florunner' (Norden *et al.,* 1969) cultivar. Unfortunately, Florunner is highly susceptible to tomato spotted wilt caused by *Tomato spotted wilt virus* (TSWV), and TSWV is also a major disease problem in the southeast U.S. So, the combination of TSWV and RKN-resistance was incorporated into the release of 'Tifguard' (Holbrook *et al.*, 2008) cultivar which used COAN as the RKN-resistant donor parent.

Since these earlier cultivar releases, molecular markers were identified for marker assisted selections (MAS) and used to develop 'Tifguard Hi-O/L' peanut (Chu *et al.*, 2011). Sequence characterized amplified region (SCAR) marker 197/909 is a new nematode resistance dominant marker (Chu *et al.*, 2007); whereas, codominant simple sequence repeat (SSR) marker GM565 (Nagy *et al.*, 2010) can be used to identify the heterozygotes for nematode resistance. The objective of the present study was to determine the accuracy of these two new molecular markers versus actual field test results for RKN-resistant screening.

Materials and Methods

During 2011 and 2012, field tests were conducted at the Rigdon Farm (latitude: 31.516° N and longitude: 83.545° W) and in 2012 at the Blackshank Farm (31.500° N and 83.545° W, respectively) near the Coastal Plain Experiment Station, Tifton, GA. Each year 15 genotypes (12 advanced Georgia breeding lines and 3 check cultivars) were evaluated at the Rigdon Farm location for RKN-resistance. The advanced breeding lines result from the same three-way cross combination ['Georgia-02C' (Branch, 2003) × ('Georgia-01R' (Branch, 2002) × COAN)], and the check cultivars were the RKN-

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resistant Tifguard and the susceptible check cultivars 'Georgia-07W' (Branch and Brenneman, 2008) and 'Georgia Greener' (Branch, 2007). Both test sites have a long history of high RKN populations and continuous peanut production. The soil type at both locations was a Tifton loamy sand (fine-loamy, kaolinitic, thermic Plinthic Kandiudults).

Planting dates at the Rigdon Farm were May 6, 2011 and May 11, 2012, and the seeding rates were six seed per 30.5-cm of row. Recommended cultural practices with irrigation were used throughout each growing season, except that no nematicides were applied with activity against RKN. A randomized complete block design was used each year with three replications. Plots consisted of two rows 7.62 m long × 1.83 m wide (0.91 m spacing between rows). The severity of nematode galling on roots and pods was rated after digging and inverting the plants each year. The percent damage was visually estimated from 0 to 100% with 0% representing no galls and 100% representing galling on all pods and roots. Leaf tissue samples from young unfolded terminal leaflets were taken from each plot early during the growing season and sent to ESTA Lab in Longmont, CO for molecular nematode screening using two markers (SCAR 197/909 and SSR-GM565). Individual plots were harvested near optimum maturity according to visual above-ground disease pressure in conjunction with the hull-scrape maturity method from adjoining border plots (Williams and Drexler, 1981). Plots were mechanically harvested, and pods were dried with forced warm air to 10% moisture content, before weighing for pod yield.

Planting date at the Blackshank Farm was April 27, 2012 at a seeding rate of six seed per 30.5-cm of row. Recommended cultural practices with irrigation were used throughout the growing season, except that no nematicides were applied with activity against RKN. A randomized complete block design was used with ten genotypes and five replications. Plots consisted of two rows 7.62 m long × 1.83 m wide (0.91 m spacing between rows). The severity of nematode galling on roots and pods was rated after digging and inverting the plants each year. The percent damage was visually estimated from 0 to 100%, with 0% representing no galls and 100% representing galling on all pods and roots. All plots were dug at the same time, Oct. 8. Plots were mechanically harvested, and pods were dried with forced warm air to 10% moisture content, before weighing for pod yield.

Reciprocal crosses were made in the fall of 2011 between nematode resistant parents (GA 082522 × Tifguard) and (GA 082524 × Tifguard). The F_1 hybrids were grown in 2012, and F_2 segregating populations were grown in the greenhouse during the winter months of 2012 to 2013. Leaf samples were taken from young immature leaflets and sent to ESTA Lab for RKN molecular marker screening using both SCAR 197/909 and SSR-GM565.

Data for all three field experiments were subjected to analysis of variance. LSD T-test was used for mean separation at the $P \leq 0.05$ probability level. Genetic analysis using molecular markers for RKN resistance was based upon individual plants, and segregation data was analyzed by chi-square program to test goodness-of-fit of observed vs. expected genetic ratios.

Results and Discussion

The 15 peanut genotypes used in this study were not present in all three field test experiments. Consequently, each test was analyzed separately, and the results from these field tests versus molecular markers will be discussed individually.

During 2011 at the Rigdon Farm, all three check cultivars were classified correctly by the two RKN molecular markers (SCAR 197/909 and SSR-GM565) with Tifguard being resistant and Georgia-07W and Georgia Greener each being susceptible (Table 1). Three of the 12 advanced Georgia breeding lines (GA 082548, GA 082549, and GA 082550-MS10) had intermediate RKN-resistance in the field. The SCAR 197/909 marker classified these three breeding lines as resistant; instead of, the SSR-GM565 marker classified the same three breeding lines as heterozygous (Table 1). In 2012 at the Rigdon Farm (Table 2), GA 082548 and GA 082549 were not significantly different ($P \leq 0.05$) from the RKN-resistant check Tifguard for gall rating and pod yield. Also in 2012 at the Blackshank Farm (Table 3), GA 082550-MS10 was again not significantly different from Tifguard for gall rating and pod yield.

However in 2011 at the Rigdon Farm, four of the 12 advanced Georgia breeding lines (GA 082511, GA 082513, GA 082514, and GA 082522) from the same cross combination had RKN-resistance in the field, but both molecular markers classified the four breeding lines as susceptible (Table 1). Thus, 26.7% among these 15 genotypes were misclassified by both molecular markers as false negatives.

Likewise in 2012 at the same Rigdon Farm field test location, the same results were found (Table 2). All three check cultivars were classified correctly by both markers, but the same four RKN-resistant breeding lines (GA 082511, GA 082513, GA

Table 1. Root-Knot Nematode Gall Rating and Pod Yield among 15 Peanut Genotypes Versus Two Molecular Markers in Field Tests at the Rigdon Farm near Tifton, GA, 2011.

	Molecular Markers			Field Results	
Peanut Genotype	SCAR 197/909	SSR GM565	(VS)	Nematode Rating	Pod Yield
				(%)	(kg/ha)
GA 082511	S[b]	S		0.0 c[a]	3309 a
GA 082545	R	H		0.0 c	2875 ab
GA 082521	R	H		0.0 c	2865 ab
GA 082513	S	S		1.0 c	2854 ab
GA 082549	R	H		21.7 b	2843 ab
GA 082514	S	S		0.0 c	2832 ab
GA 082546	R	R		0.7 c	2713 abc
GA 082519	R	H		0.0 c	2680 abc
GA 082524	R	R		1.7 c	2583 abc
GA 082522	S	S		0.0 c	2528 abc
GA 082550-MS10	R	H		31.7 b	2495 abc
Tifguard (ck)[b]	R	R		0.0 c	2409 abc
GA 082548	R	H		23.3 b	2192 abc
Georgia Greener (ck)[b]	S	S		56.7 a	1682 bc
Georgia-07W (ck)[b]	S	S		51.7 a	1410 c

[a]Within columns, means followed by the same letter are not significantly different at P≤0.05.
[b]R = resistant, S = susceptible, H = heterozygous, and (ck) = check cultivar.

082514, and GA 082522) were again misclassified as susceptible by both molecular markers.

In 2012 at the Blackshank Farm, similar results were also observed (Table 3). This field test location involved fewer genotypes but more replications. The resistant check cultivar Tifguard and several entries of the susceptible check cultivar Georgia-07W were again in agreement with both markers. However, GA 082522 again showed RKN-resistant in the field test but was misclassified as susceptible by both markers.

Only five of the 12 advanced Georgia breeding lines (GA 082519, GA 082521, GA 082524, GA 082545, and GA 082546) were classified as resistant

Table 2. Root-Knot Nematode Gall Rating and Pod Yield among 15 Peanut Genotypes Versus Two Molecular Markers in Field Tests at the Rigdon Farm near Tifton, GA, 2012.

	Molecular Markers			Field Results	
Peanut Genotype	SCAR 197/909	SSR GM565	(VS)	Nematode Rating	Pod Yield
				(%)	(kg/ha)
GA 082514	S[b]	S		0.7 e[a]	5990 a
GA 082522	S	S		0.8 de	5632 a
GA 082519	R	H		0.0 e	5578 a
GA 082524	R	R		0.0 e	5437 a
GA 082511	S	S		0.0 e	5219 a
GA 082545	R	H		0.8 de	5208 a
GA 082521	R	H		0.0 e	5208 a
GA 082513	S	S		0.0 e	5208 a
GA 082546	R	R		0.0 e	5133 ab
GA 082549	R	H		9.2 cd	4786 abc
Tifguard (ck)[b]	R	R		4.6 cde	4688 abc
GA 082548	R	H		12.9 c	3809 bcd
GA 082550-MS10	R	H		22.9 b	3668 cde
Georgia-07W (ck)[b]	S	S		74.2 a	3136 de
Georgia Greener (ck)[b]	S	S		76.7 a	2365 e

[a]Within columns, means followed by the same letter are not significantly different at P≤0.05.
[b]R = resistant, S = susceptible, H = heterozygous, and (ck) = check cultivar.

Table 3. Root-Knot Nematode Gall Rating and Pod Yield among Ten Peanut Genotypes Versus Two Molecular Markers in Field Tests at the Blackshank Farm near Tifton, GA, 2012.

	Molecular Markers			Field Results	
Peanut Genotype	SCAR 197/909	SSR GM565	(VS)	Nematode Rating	Pod Yield
				(%)	(kg/ha)
GA 082524	R[b]	R		0.0 e[a]	4349 a
GA 082522	S	S		0.0 e	3835 ab
GA 082546	R	R		0.0 e	3249 b
Tifguard (ck)[b]	R	R		1.2 de	3060 bc
GA 082550-MS10	R	H		5.8 d	2845 bcd
Georgia-07W (ck)[b]	S	S		35.0 b	2200 cde
Georgia-07W (ck)[b]	-	-		34.7 b	2155 cde
Georgia-07W (ck)[b]	-	-		27.9 c	1999 de
Georgia-07W (ck)[b]	-	-		30.6 bc	1804 e
Georgia-07W (ck)[b]	-	-		40.9 a	1771 e

[a]Within columns, means followed by the same letter are not significantly different at P≤0.05.
[b]R = resistant, S = susceptible, H = heterozygous, and (ck) = check cultivar.

by at least one marker and were found to have very low nematode gall rating and relatively high pod yields (Tables 1 and 2). Two of these breeding lines (GA 082524 and GA 283546) were consistently and correctly classified as RKN-resistant each year by both molecular markers in all three field tests (Tables 1–3).

These findings suggest genetic differences among the MAS resistant and MAS susceptible genotypes. Reciprocal cross combinations between MAS resistant Tifguard × MAS susceptible GA 082522 and between MAS resistant Tifguard × MAS resistant GA 082546 resulted in all F_1 hybrid plants testing resistant by the SCAR 197/909 marker; whereas, F_1 plants were classified as heterozygous as expected with the co-dominant SSR-GM565 marker. So, no maternal or cytoplasmic effects were observed among these reciprocal crosses in the F_1 generation.

The F_2 segregation from the reciprocal cross MAS resistant Tifguard × MAS susceptible GA 082522 fit a 3:1 resistant to susceptible ratio using the SCAR 197/909 marker (Table 4). Likewise, the F_2 segregation from the same reciprocal cross fit a 1:2:1 ratio using the SSR-GM 565 marker (Table 5). Total, combined, and homogeneity chi-squared values also agreed with the one-gene genetic ratio of either 3:1 vs 1:2:1 depending upon the molecular marker utilized. These results suggest one-gene difference among these two MAS parental genotypes (Tifguard vs GA 082522).

No F_2 segregation was observed in the reciprocal cross of MAS resistant Tifguard × MAS resistant GA 082546. This would be expected since both parental MAS resistant genotypes were classified correctly by these two molecular markers.

Conclusions

The one-gene difference found in the MAS resistant × MAS susceptible reciprocal cross combinations suggest that genetic recombination may have occurred between the two molecular markers (SCAR 197/909 and SSR-GM565) and the RKN-resistant gene, *Rma.* This lack of linkage could

Table 4. F_2 Plant Segregation for Root-Knot Nematode Resistance using the Molecular Marker SCAR 197/909 among a Reciprocal MAS Peanut Cross Combination.

	No. of Plants		χ^2	
Cross	Resist.	Suscept.	(3:1)	ρ
GA 082522 × Tifguard	70	28	0.667	0.25–0.50
Tifguard × GA 082522	71	35	3.635	0.05–0.10
Total			4.302	0.10–0.25
Pooled	141	63	3.765	0.05–0.10
Homogeneity			0.537	0.25–0.50

Table 5. F_2 Plant Segregation for Root-Knot Nematode Resistance using the Molecular Marker SSR-GM565 among a Reciprocal MAS Peanut Cross Combination.

	No. of Plants			χ^2	
Cross	Resist.	Hetero.	Suscept.	(1:2:1)	ρ
GA 082522 × Tifguard	22	58	29	1.349	0.50–0.75
Tifguard × GA 082522	20	51	37	5.686	0.05–0.10
Total				7.035	0.10–0.25
Pooled	42	109	66	5.313	0.05–0.10
Homogeneity				1.722	0.25–0.50

explain the misclassification among four of the 15 genotypes that were found in the three field tests conducted during this 2-yr study (Tables 1 to 3).

Another possibility may also be the identification of a second unlinked nematode resistant gene. Garcia *et al.*, (1996) first proposed two dominant genes, *Mae* and *Mag*, conditioning peanut root-knot nematode resistance from a F_2 population derived from the interspecific cross of 4× (*A. hypogaea X A. cardenasii*) = GA 6 and PI 261942. Yet, another possibility may be attributed to random chromosome segregation involving an autotetraploid introgressed large segment with the RKN-resistant gene(s) from the original 3-way [BB × (AA × AA) genome] cross combination as described by Strickberger (1968).

Regardless of the reason, greater than a fourth of the RKN-resistant peanut genotypes would have been missed by using only these two molecular markers in a MAS breeding program. Consequently, the need for molecular markers more tighter-linked with the RKN-resistant gene(s) is now apparent for future marker assisted selection.

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