

Evidence for a Second RKN Resistance Gene in Peanut

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ABSTRACT

Root-knot nematode (RKN), [*Meloidogyne arenaria* (Neal) Chitwood race 1] can result in highly significant yield losses in peanut (*Arachis hypogaea* L.) production. Fortunately, very high levels of RKN nematode resistance have been identified and incorporated from wild species into newly developed peanut cultivars. In 2011-12 at Tifton, GA, a field site was artificially inoculated with *M. arenaria* race 1. A susceptible cultivar was used to uniformly increase the peanut-specific race 1 nematode population during the summer and fall; whereas, hairy vetch (*Vicia villosa* Roth) was used for the same purpose each winter as a susceptible cover crop. During 2013 and 2014, space-planted F₂ and F₃ populations from cross combinations involving *A. hypogaea* susceptible × resistant parental lines derived from ‘COAN’ were evaluated, respectively. Several past inheritance studies had suggested a single dominant gene, *Rma*, controlled the resistance. However in this study, the occurrence of a second recessive gene (*rma*₂) was also found to be involved in very high peanut RKN resistance. Inheritance data fit a 13:3 genetic model and confirmed an earlier report for two RKN-resistance genes (*Rma*₁ and *rma*₂) found in TxAG-6 and now COAN.

Key Words: *Arachis hypogaea* L., groundnut, inheritance data, *Meloidogyne arenaria* (Neal) Chitwood race 1, genetic ratio.

Root-knot nematodes (RKN) [*Meloidogyne arenaria* (Neal) Chitwood race 1] are a costly peanut (*Arachis hypogaea* L.) production problem in the U.S. (Kokalis-Burelle and Rodriguez-Kabana 1997; Starr *et al.*, 2002). Introgression of RKN resistance from the wild species resulted in the first improved peanut cultivar ‘COAN’ (Simpson and Starr 2001) with a very high RKN resistance. Since then, several other RKN-resistant cultivars have been developed from COAN:

‘NemaTAM’ (Simpson *et al.* 2003), ‘Webb’ (Simpson *et al.* 2013); ‘Tifguard’ (Holbrook *et al.* 2008); ‘Georgia-14N’ (Branch and Brenneman 2015), and ‘TifNV-High O/L’.

Inheritance of the very high RKN resistance found in COAN was first reported to be controlled as a single dominant gene in several studies (Burow *et al.* 1996; Choi *et al.* 1999; Chu *et al.* 2011; Church *et al.* 2000). Nagy *et al.* (2010) proposed the name *Rma* for this single dominant RKN resistance gene.

In 1996, Garcia *et al.* suggested two dominant genes (*Mae* and *Mag*) controlling egg number and galling, respectively. However, the F₂ data were derived from different parental material than COAN. In yet another genetic study by Church *et al.* (2005), a recessive gene in addition to the single dominant gene was also proposed involving TxAG-6 (Simpson *et al.*, 1993), the interspecific hybrid that served as the source of RKN resistance. The objective of current genetic study was to further determine the inheritance of a proposed second RKN resistance gene found in peanut derived from COAN.

Materials and Methods

During 2011 and 2012, a new field site at the Gibbs Farm (latitude: 31.4312°N and longitude: 83.5850°W) near the Coastal Plain Experiment Station, Tifton, GA was artificially inoculated with *M. arenaria* race 1. The susceptible peanut cultivar, ‘Georgia-10T’ (Branch and Culbreath 2011) was used to uniformly increase the peanut-specific race 1 nematode population during the growing season; whereas, hairy common vetch (*Vicia villosa* Roth) was used for the same purpose each winter as a susceptible cover crop.

Crosses were made in the greenhouse between the RKN-susceptible parent ‘Georgia Greener’ (Branch 2007) and two advanced RKN-resistant Georgia breeding lines, GA 082524 and GA 082546 (Branch *et al.* 2014). These two homozygous breeding lines resulted from the same three-way cross combination ‘Georgia-02C’ (Branch 2003) × [‘Georgia-01R’ (Branch 2002) × COAN].

Seed of the F₂ and F₃ cross populations were space-planted 30.5-cm apart during 2013 and 2014, respectively in the newly RKN inoculated field site. Planting dates were 22 May 2013 and 23 May 2014. Recommended cultural practices with irrigation

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Table 1. F₂ plant segregation and χ^2 analyses for root-knot nematode (RKN) susceptible and resistant plants from two peanut cross combinations when grown in field test with high RKN populations, 2013.

Cross Combination	F ₂ Plant segregation		χ^2		χ^2	
	Resistant	Susceptible	(3:1)	P	(13:3)	P
Georgia Greener × GA 082524	102	22	3.484	0.062	0.083	0.774
Georgia Greener × GA 082546	100	21	3.771	0.052	0.154	0.694
Total			7.255	<0.050	0.236	0.894
Pooled	202	43	7.250	<0.050	0.231	0.631
Homogeneity			0.005	0.945	0.005	0.945

were used throughout each growing season, except that no nematicides were applied with activity against RKN. The severity of nematode galling on roots and pods was rated after digging and inverting the plants each year. All plants were dug at the same time on 21 Oct 2013 and 29 Oct 2014. The percent damage was visually estimated from 0 to 100%, with 0% representing no galls and 100% representing galling on all pods and roots. Since there can sometimes be a low level of gall formation on resistant plants, a 5% level of galling was used as a threshold in defining resistant and susceptible genotypes. Data from visual classification of segregating RKN-resistant and RKN-susceptible plants and progeny row were analyzed by a chi-square program to test goodness-of-fit of observed vs expected genetic ratios.

Results and Discussion

During 2013, the F₂ plant segregation showed an acceptable fit to both a 3:1 and 13:3, resistant to susceptible genetic ratios, respectively (Table 1). However, the 13:3 ratio had the best fit with the lowest chi-square values and highest probability compared to the 3:1 ratio.

Because of the number of genes inherited, one of the major differences between a 3:1 vs 13:3 ratio is that the F₂ susceptible plants should not segregate for resistance and susceptible plants from a 3:1 ratio in the following F₃ generation; whereas, the F₂ susceptible plants would segregate from the 13:3 ratio. Both resistant and susceptible plants were found segregating within some of the F_{2:3} susceptible progeny rows in a 1 resistant to 3 susceptible ratio.

The F₃ progeny row segregation showed an acceptable fit to a 6 segregating to 7 non-segregating ratio for the F_{2:3} RKN-resistant plants from the Georgia Greener x GA 082524 cross combination (Table 2). Likewise, the F₃ progeny row segregation from the F_{2:3} RKN-susceptible plants was found to have an acceptable fit to a 2 segregating to 1 non-segregating ratio as expected for a 13:3 genetic model.

Table 2. F₃ progeny row segregation and χ^2 analyses from F₂ root-knot nematode (RKN) resistant and susceptible peanut plants from the Georgia Greener × GA 082524 cross combination when grown in field test with high RKN population, 2014.

F ₂ Plants	F _{2:3} Prog. row segregation		Exp. ratio	χ^2	P
	Seg.	None			
Resistant	23	16	(6:7)	2.581	0.108
Susceptible	11	8	(2:1)	0.658	0.417

The evidence from this study strongly confirms the report by Church *et al.* (2005). The first RKN-resistant gene should be dominant as previously reported, designated *Rma*₁. However, the second RKN-resistance gene should be recessive with a proposed gene symbol, *rma*₂. As expected among segregating F₂ plants and F_{2:3} progeny rows in a 13:3 ratio from a two-gene model (Table 3), either the homozygous or heterozygous dominant gene *Rma*₁ *Rma*₁ or *Rma*₁ *rma*₁, homozygous recessive gene *rma*₂ *rma*₂, or the various combinations of these two genes results in the very high level of peanut - RKN resistance.

Table 3. An expected 13:3 ratio from a two-gene model of F₂ plant and F_{2:3} progeny row segregation for peanut RKN resistance.

Exp. ratio	F ₂ Genotype	F ₂ Phenotype	F _{2:3} Prog. row seg.
1	<i>Rma</i> ₁ <i>Rma</i> ₁ <i>Rma</i> ₂ <i>Rma</i> ₂	Resistant	All R ^a
2	<i>Rma</i> ₁ <i>Rma</i> ₁ <i>Rma</i> ₂ <i>rma</i> ₂	Resistant	All R
1	<i>Rma</i> ₁ <i>Rma</i> ₁ <i>rma</i> ₂ <i>rma</i> ₂	Resistant	All R
2	<i>Rma</i> ₁ <i>rma</i> ₁ <i>Rma</i> ₂ <i>Rma</i> ₂	Resistant	Seg. (3R:1S)
4	<i>Rma</i> ₁ <i>rma</i> ₁ <i>Rma</i> ₂ <i>rma</i> ₂	Resistant	Seg. (13R:3S)
2	<i>Rma</i> ₁ <i>rma</i> ₁ <i>rma</i> ₂ <i>rma</i> ₂	Resistant	All R
1	<i>rma</i> ₁ <i>rma</i> ₁ <i>Rma</i> ₂ <i>Rma</i> ₂	Susceptible	All S
2	<i>rma</i> ₁ <i>rma</i> ₁ <i>Rma</i> ₂ <i>rma</i> ₂	Susceptible	Seg. (1R:3S)
1	<i>rma</i> ₁ <i>rma</i> ₁ <i>rma</i> ₂ <i>rma</i> ₂	Resistant	All R

^aAbbreviations: R, Resistant; S, Susceptible for RKN.

Literature Cited

- Branch, W.D. 2002. Registration of 'Georgia-01R' peanut. *Crop Sci.* 42:1750-1751.
- Branch, W.D. 2003. Registration of 'Georgia-02C' peanut. *Crop Sci.* 43:1883-1884.
- Branch, W.D. 2007. Registration of 'Georgia Greener' peanut. *J. Plant Reg.* 1:121.
- Branch, W.D. and T.B. Brenneman. 2015. Registration of 'Georgia-14N' peanut. *J. Plant Reg.* 9:159-161.
- Branch, W.D. and A.K. Culbreath. 2011. Registration of 'Georgia-10T' peanut. *J. Plant Reg.* 5:279-281.
- Branch, W.D., T.B. Brenneman, and G. Hookstra. 2014. Field test results versus marker assisted selection for root-knot nematode resistance in peanut. *Peanut Sci.* 41:85-89.
- Burow, M.D., C.E. Simpson, A.H. Paterson, and J.L. Starr. 1996. Identification of peanut (*Arachis hypogaea* L.) RAPD markers diagnostic of root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood) resistance. *Mol. Breeding* 2:369-379.
- Choi, K., M.D. Burow, G. Church, G. Burow, A.H. Paterson, C.E. Simpson, and J.L. Starr. 1999. Genetics and mechanism of resistance to *Meloidogyne arenaria* in peanut germplasm. *J. Nematology* 31:283-290.
- Church, G.T., C.E. Simpson, M.D. Burow, A.H. Paterson, and J.L. Starr. 2000. Use of RFLP markers for identification of individuals homozygous for resistance to *Meloidogyne arenaria* in peanut. *Nematology* 2:575-580.
- Church, G.T., J.L. Starr, and C.E. Simpson. 2005. A recessive gene for resistance to *Meloidogyne arenaria* in interspecific *Arachis* spp. hybrids. *J. Nematology* 37:178-184.
- Chu, Y., C.L. Wu, C.C. Holbrook, B.L. Tillman, G. Person, and P. Ozias-Akins. 2011. Marker-assisted selection to pyramid nematode resistance and the high oleic trait in peanut. *The Plant Genome.* 4:110-117.
- Garcia, G.M., H.T. Stalker, E. Shroeder, and G. Kochert. 1996. Identification of RAPD, SCAR, and RFLP markers tightly linked to nematode resistance genes introgressed from *Arachis cardenasii* into *Arachis hypogaea*. *Genome* 39:836-845.
- Holbrook, C.C., P. Timper, A.K. Culbreath, and C.K. Kvien. 2008. Registration of 'Tifguard' peanut. *J. Plant Reg.* 2:92-94.
- Kokalis-Burelle, N. and R. Rodriguez-Kabana. 1997. Root-knot nematodes. In: *Compendium of Plant Diseases 2nd Ed.*, Amer. Phytopath. Soc. Press, pp. 45-48.
- Nagy, E.D., Y. Chu, Y. Guo, S. Khanal, S. Tang, Y. Li, W.B. Dong, P. Timper, C. Taylor, P. Ozias-Akins, C.C. Holbrook, V. Beilinson, N.C. Nielson, H.T. Stalker, and S.J. Knapp. 2010. Recombination is suppressed in an alien introgression in peanut harboring *Rma*, a dominant root-knot nematode resistance gene. *Mol. Breeding* 26:357-370.
- Simpson, C.E. and J.L. Starr. 2001. Registration of 'COAN' peanut. *Crop Sci.* 41:918.
- Simpson, C.E., S.C. Nelson, J.L. Starr, K.E. Woodard, and O.D. Smith. 1993. Registration of Tx-AG-6 and TxAG-7 peanut germplasm lines. *Crop Sci.* 33:1418.
- Simpson, C.E., J. L. Starr, G.T. Church, M.D. Burow, and A.H. Paterson. 2003. Registration of 'NemaTAM' peanut. *Crop Sci.* 43:1561.
- Simpson, C.E., J. L. Starr, M.R. Baring, M.D. Burow, J.M. Cason, and J.N. Wilson. 2013. Registration of 'Webb' peanut. *J. Plant Reg.* 7:265-268.
- Starr, J.L., J. Bridge, and R. Cook. 2002. Resistance to plant-parasitic nematodes: history, current use and future potential. Pp: 1-22 In: J. L. Starr, R. Cook, and J. Bridge (eds). *Plant Resistance to Parasitic Nematodes*. CABI Publishing, CAB International, New York, NY.