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A Recessive Gene for Resistance to *Meloidogyne arenaria* in Interspecific *Arachis* spp. Hybrids

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Abstract: A single dominant gene for resistance to *Meloidogyne arenaria* was identified previously in two peanut cultivars, *Arachis hypogaea* 'COAN' and 'NemaTAM'. The interspecific *Arachis* hybrid TxAG-6 was the source of this resistance and the donor parent in a backcross breeding program to introgress resistance into cultivated peanut. To determine if other resistance genes were present in TxAG-6 and derived breeding populations from the third backcross generation (BC_3), F_2 individuals were evaluated for the resistance phenotype. The ratio of the resistant and susceptible individuals for all F_2 populations fit the expected ratio for resistance being governed by one dominant gene and one recessive gene. Evaluation of the F_3 generation from four susceptible F_2 individuals (two from TxAG-6 × *A. hypogaea* and two from the BC_3 population) confirmed that a recessive gene for resistance to *M. arenaria* was present in each of the tested populations. The identification of a second gene for resistance in the *A. hypogaea* germplasm may improve the durability of the resistance phenotype.

Key words: Arachis hypogaea, durable resistance, *Meloidogyne arenaria,* peanut, recessive inheritance, resistance genes, root-knot nematode.

The discovery of resistance to *Meloidogyne arenaria* and *M. javanica* in wild *Arachis* species and the interspecific hybrid, TxAG-6, allowed the development of the first peanut cultivar resistant to *M. arenaria* (Nelson et al., 1989; Simpson, 1991; Simpson et al., 1993; Simpson and Starr, 2001). This resistance provides an improved method of managing root-knot nematodes in peanut production. The durability of this monogenic resistance is unknown, however, and can be determined only after it is used over an extended period of time and across a large geographical area.

The extensive use of resistance to *Heterodera glycines* in soybean has resulted in the selection of *H. glycines* populations virulent on the resistant cultivars (Young, 1992). Similarly, populations of *M. incognita* and *M. javanica* able to reproduce on tomato plants carrying the *Mi* resistance gene have been discovered in California and Spain, respectively (Kaloshian et al., 1996; Ornat et al., 2001). Durability of resistance has been shown to be enhanced when multiple genes for resistance are used together or in a deployment strategy designed to reduce selection for virulence (Johnson, 1984; Wilson et al., 2001).

In all advanced generation populations, including the cultivars COAN and NemaTAM, in a breeding program to introgress resistance from TxAG-6 (*A. batizocoi* × [*A. diogoi* × *A. cardenasii*])$^{4\times}$ to peanut cultivars, resistance was inherited as a single dominant gene (Choi et al., 1999; Church et al., 2000). This resistance gene was derived from the A genome parent *A. cardenasii* (Burow et al., 1996).

Data suggest the existence of additional resistance genes in the synthetic tetraploid TxAG-6. The different mechanisms of resistance that exist in the wild *Arachis* species suggest different genes for resistance exist in the different wild species (Nelson et al., 1990; Starr et al., 1990). Each of the three parental species of TxAG-6 was resistant to *M. arenaria,* and each could have contributed a separate resistance gene (Nelson et al., 1989; Simpson, 1991). Segregation patterns in the F_2 generation from the cross between *A. cardenasii* and the susceptible *A. duranensis* suggested that *A. cardenasii* possessed at least two dominant resistance genes (Starr and Simpson, 1991). In studies involving an interspecific cross between *A. cardenasii* and *A. hypogaea,* two dominant genes for nematode resistance were identified (Garcia et al., 1996; Stalker et al., 1995). Collectively, these observations support the hypothesis that additional genes for resistance to *M. arenaria* derived from the wild *Arachis* species may have been introgressed into TxAg-6.

The objective of this study was to examine inheritance of resistance in two early generation breeding populations derived from TxAg-6 to determine if additional genes for resistance are present. The identification of additional resistance genes in peanut germplasm may enhance the durability of resistance to *M. arenaria.*

Materials and Methods

Resistance evaluations: The F_2 progeny of *A. hypogaea* 'Florunner' (susceptible) × TxAg-6 (resistant), and a third backcross (BC_3) generation population derived from the same cross with Florunner as the recurrent parent, were evaluated for resistance to *M. arenaria* (Fig. 1). There was no selection for nematode resistance or agronomic traits during the development of the BC_3 population. Seeds from the F_2 and F_3 generations from the cross between the susceptible *A. hypogaea* and the TxAG-6 were treated with captan (Gustafson, Plano, TX) and germinated on rolled germination paper wetted with a 21.7% solution (v/v) of etheryl (Union Carbide, Research Triangle Park, NC) and in-

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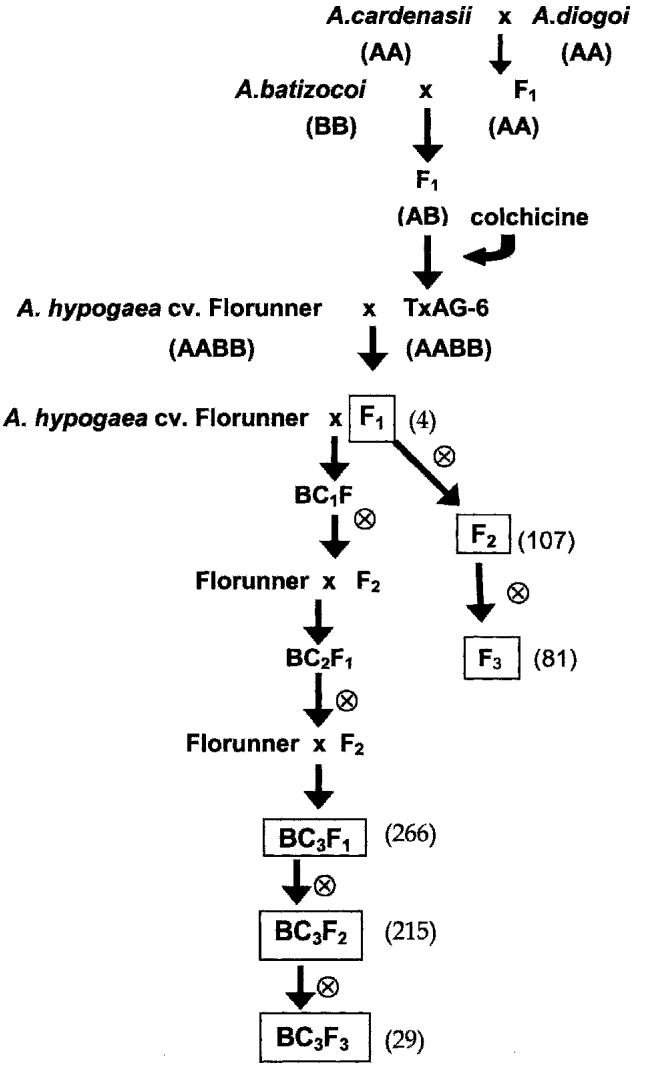

FIG. 1. The crossing scheme used to create TxAG-6 and backcross breeding populations. The numbers of individuals tested for the resistance phenotype in this study are listed in parentheses. ⊗ indicates that generation was self-pollinated.

cubated at 25 °C for 7 days. The germinated seeds were placed in 13-cm-diam. pots filled with a coarse sand-peat soil mix (6:1, v/v) that were then placed in a greenhouse for resistance evaluation.

A BC_3F_1 population of 474 individuals was propagated vegetatively in a greenhouse. The RFLP locus R2430E was used to identify the putative genotype with respect to the known dominant resistance gene for each individual as described by Choi et al. (1999) and Church et al. (2000). Three stem cuttings were taken from each plant that was identified as lacking the R2430E RFLP locus. Cuttings were treated with rooting hormone (0.1% indole-3 butyric acid; Green Light Company, San Antonio, TX), placed in peat pellets, and then incubated in a growth chamber at 28 °C for 4 weeks. Once the cuttings had developed adventitious roots, the peat pellets were removed and the cuttings were planted in 13-cm-diam. pots filled with a coarse sand-peat soil mix (6:1, v/v) for resistance evaluation.

Inoculum for resistance screening was prepared by extracting eggs of *M. arenaria* isolate #92-6 from roots of infected tomato plants with 0.052% NaOCl (Hussey and Barker, 1973). Individual plants were inoculated 3 weeks after being placed in a pot by pipeting equal amounts of a suspension containing 10,000 eggs into three holes in the soil, equidistant from each other. The inoculated plants were maintained in a greenhouse at 25 to 32 °C and fertilized with a slow-release fertilizer containing 14-7-7 (N-P-K) and micronutrients (Sierra Mix, Milpitas, CA). Plants were removed from their pots 8 weeks after inoculation, and the soil was washed from the roots with water. The roots were blotted dry and a 5-g root sample was collected for nematode egg extraction using 1.04% NaOCl (Hussey and Barker, 1973). The number of eggs per gram of fresh root was determined for each individual. Individual plants that had less than 10% of the mean number of eggs per gram of root for the susceptible parent Florunner were categorized as resistant (Starr et al., 1995).

BC_3F_1 individuals that were identified in the initial greenhouse tests as resistant to *M. arenaria* but lacking the resistance locus identified by RFLP marker R2430E were propagated by stem cuttings and transplanted into field microplots. Microplots consisted of plastic cylinders (55 cm diam. × 45 cm deep) filled and buried in a loamy sand soil (85% sand, 2% silt, and 8% clay; pH ~7.5). Prior to planting and inoculation with soil infested with *M. arenaria,* each plot was fumigated with 1,3-dichloropropene to eliminate existing pathogen populations. Inoculation of *M. arenaria* was performed as reported by Abdel-Momen and Starr (1997), with an inoculum density of 400 eggs and second-stage juveniles/500 cm^3 of soil for each plot. Additional microplots were planted with the resistant control COAN and the susceptible control Florunner. Plants were grown to maturity and seed was harvested. The number of eggs per gram of fresh root was determined as described by Starr et al. (1995). The RFLP loci R2430E and S1018E (Church, 2002) were used to determine the presence of the linked resistance locus for individuals identified as resistant from microplot tests. These F_2 individuals were screened in a greenhouse test for resistance to *M. arenaria.*

Four arbitrarily selected F_2 individuals, two from the

TABLE 1. Chi-square analysis of observed segregation of resistance to *Meloidogyne arenaria* in the F_2 generation from the cross *Arachis hypogaea* cv. Florunner × TxAG-6.

Genetic model	Expected ratio (R:S)	Observed		Expected		χ^2
		R	S	R	S	
XX	(3:1)	92	15	80.3	26.7	6.88*
XXYY	(15:1)	92	15	100.3	6.7	11.02*
XXyy	(13:3)	92	15	86.9	20.1	1.57

* Deviates from the expected value ($P = 0.05$); critical value is 3.84.

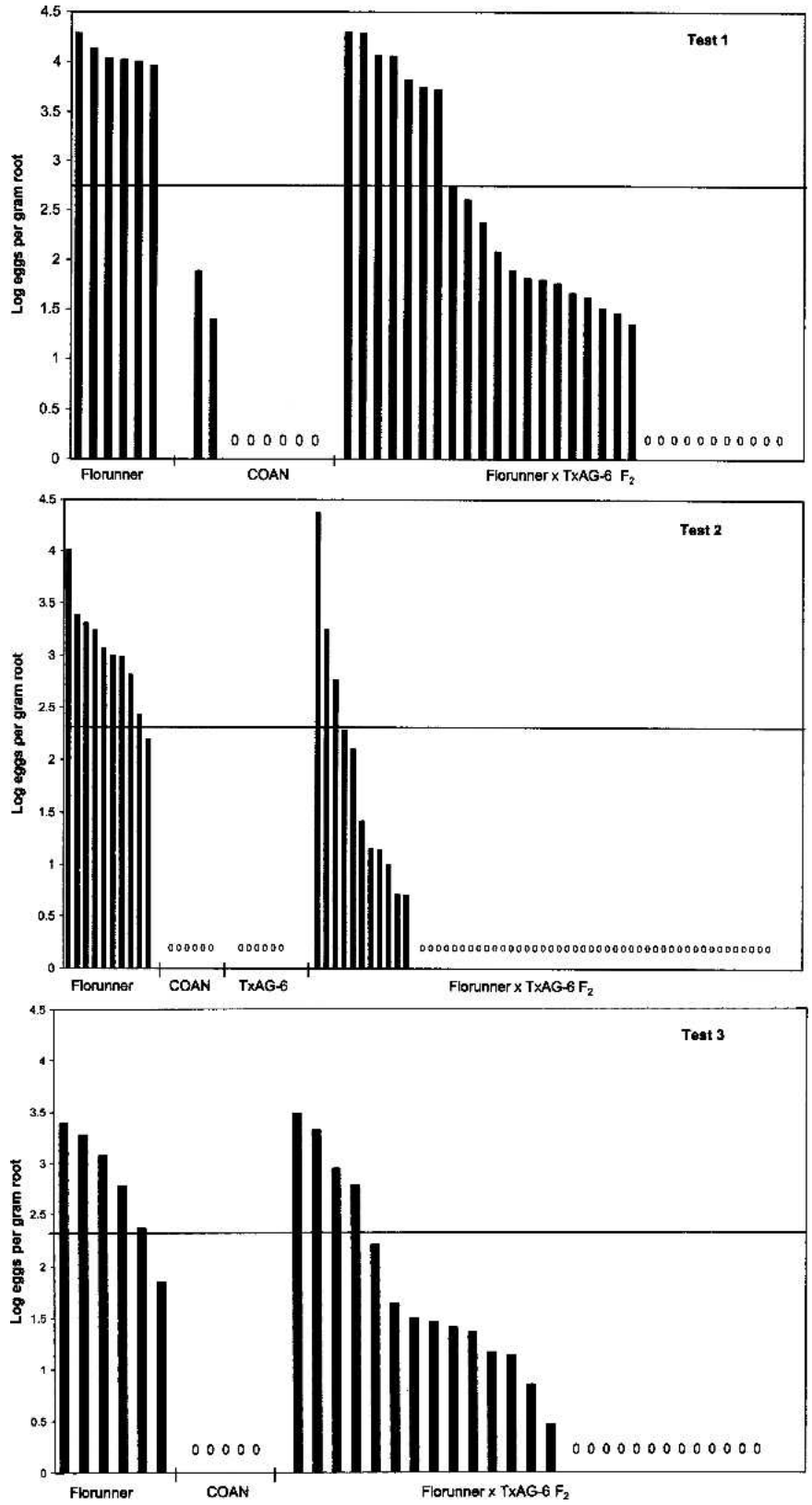

FIG. 2. Egg production per gram root of *Meloidogyne arenaria* on 107 individual F_2 plants from the cross between *Arachis hypogaea* cv. Florunner × TxAG-6. Data are given for three tests. The horizontal lines represent 10% of the mean numbers of eggs per gram root produced on the susceptible cultivar Florunner. COAN and TxAG-6 are resistant controls. Individuals with no eggs detected are marked with 0.

TxAg-6 × Florunner population and two from the BC_3 population, that were identified as susceptible to *M. arenaria* were propagated as cuttings to produce an F_3 generation from each individual. The F_3 individuals were then screened for resistance to *M. arenaria* in 13-cm-diam. pots.

Statistical analysis: Multiple tests were performed, and data were combined for Chi-square analysis. Analysis of variance was performed on a log transformation of the eggs per gram root data using SAS 9.0 (SAS Institute, Cary, NC). Chi-square analysis was used to determine if observed ratios of resistant and susceptible individuals were different ($P = 0.05$) from expected ratios for a trait being governed by a single dominant gene (3:1, resistant [R]: susceptible [S]), two dominant genes (15:1, R:S), one dominant gene and one recessive gene (13:3, R:S), or one recessive gene (1:3, R:S).

Results

Inheritance of resistance in A. hypogaea Florunner × TxAG-6: The first test had a mean of 12,296 and 13 eggs/g root for Florunner and COAN, respectively. The second test had a mean of 2,056 and 0 eggs/g root for Florunner and COAN, respectively. The third test had a mean of 1,074 and 0 egg/g root for Florunner and COAN, respectively. In all tests, a level of reproduction less than 10% of mean reproduction of the susceptible control, Florunner, gave a clear separation of resistant and susceptible individuals. The phenotypic data from three individual tests were combined. The ratio of resistant to susceptible individuals in the F_2 generation of *A. hypogaea* Florunner × TxAG-6 was 92:15 (R:S) (Table 1; Fig. 2). This observed ratio of resistant to susceptible individuals fit the expected values for one dominant gene and one recessive gene (13:3, R:S) with a chi-square value of 1.57 ($P = 0.05$). The observed ratio did not fit the expected ratio for either a single dominant gene or two dominant genes.

Inheritance of resistance in a BC_3F_1 population: Three BC_3F_1 individuals (designated 9-1, 9-3, and 1-10) were determined in multiple greenhouse experiments to be resistant to *M. arenaria* with means of 93, 49, and 34 eggs/g root, respectively. In subsequent microplot experiments 9-1, 9-3, and 1-10 were confirmed to be resistant to *M. arenaria* with a mean final nematode density of 291, 1,215, and 2,025 eggs/g root, respectively. In these microplot tests Florunner supported a mean of 41,366 eggs/g root, whereas COAN supported a mean of 50 eggs/g root.

DNA from the three resistant BC_3F_1 individuals was analyzed with RFLP markers R2430E and S1018E (Choi, et al. 1999; Church et al., 2000, 2001). Church et al. (2001) determined that R2430E and S1018E flanked the dominant resistance gene locus with linkage distances of 1.2 and 1.8 cM, respectively, to the resistance locus. The individual 1-10 lacked the DNA fragments for both R2430E and S1018E associated with resistance. The other two individuals lacked the DNA fragment for R2430E associated with resistance but possessed the fragment for S1018E associated with resistance.

Populations of F_2 individuals from these three BC_3F_1 plants (9-1, 9-3, and 1-10) were evaluated for resistance to *M. arenaria* in the greenhouse to determine segregation of resistance. The observed ratio of resistant to susceptible individuals of the F_2 progeny from BC_3F_1 plant 9-1 was 71:10 (R:S) (Table 2; Fig. 3). This observed ratio was not different based on chi-square from the expected ratios for one dominant gene and one recessive gene (13:3, R:S). The observed ratio of resistant to susceptible individuals of the F_2 progeny from BC_3F_1 plant 9-3 was 64:8 (R:S), which fit the expected ratio for one dominant gene and one recessive gene (13:3, R:S) (Table 2; Fig. 3). However, the ratio of 64:8 (R:S) for this population also fit the expected ratio of 15:1 (R:S) for two dominant genes. The observed ratio of resistant to susceptible individuals of the F_2 progency from BC_3F_1 plant 1-10 was 49:13 (R:S) (Table 2; Fig. 3). This observed ratio was not different from the expected ratio for a single dominant (3:1, R:S) and the expected ratio for one dominant gene and one recessive gene (13:3, R:S).

Inheritance of resistance in F_3 families: To test further the hypothesis that a second, recessive resistance gene was present in TxAG-6, the F_3 generation from four susceptible F_2 individuals was tested for resistance to *M. arenaria*. Although resistant individuals were identified in two F_3 populations derived from the four susceptible *A. hypogaea* Florunner × TxAG-6 F_2 plants, the segregation ratio for the F_3 individuals did not fit the expected ratio of 1:3 (R:S) for the presence of one recessive gene (Table 3; Fig. 4). The F_3 progenies from two susceptible BC_3F_2 individuals fit the 1:3 (R:S) segregation ratio, indicating that each BC_3F_2 parent plant was heterozygous for a recessive gene (Table 3; Fig. 4).

Discussion

The ratio of resistant to susceptible individuals in the F_2 generation of the cross between Florunner and

Table 2. Chi-square analysis of segregation for resistance to *Meloidogyne arenaria* in the F_2 generation from three BC_3 populations of the cross *Arachis hypogaea* cv. Florunner × TxAG-6.

Genetic model	Expected ratio (R:S)	Observed		Expected		χ^2
		R	S	R	S	
			BC_3F_2 9-1			
XX	(3:1)	71	10	60.7	20.3	6.92*
XXYY	(15:1)	71	10	75.9	5.1	5.14*
XXyy	(13:3)	71	10	65.8	15.2	2.18
			BC_3F_2 9-3			
XX	(3:1)	64	8	54.0	18.0	7.41*
XXYY	(15:1)	64	8	67.5	4.5	2.90
XXyy	(13:3)	64	8	58.5	13.5	2.76
			BC_3F_2 1-10			
XX	(13:1)	49	13	46.5	15.5	0.54
XXYY	(15:1)	49	13	58.1	3.9	22.92*
XXyy	(13:3)	49	13	50.4	11.6	0.20

* Deviates from the expected value ($P = 0.05$); critical value is 3.84.

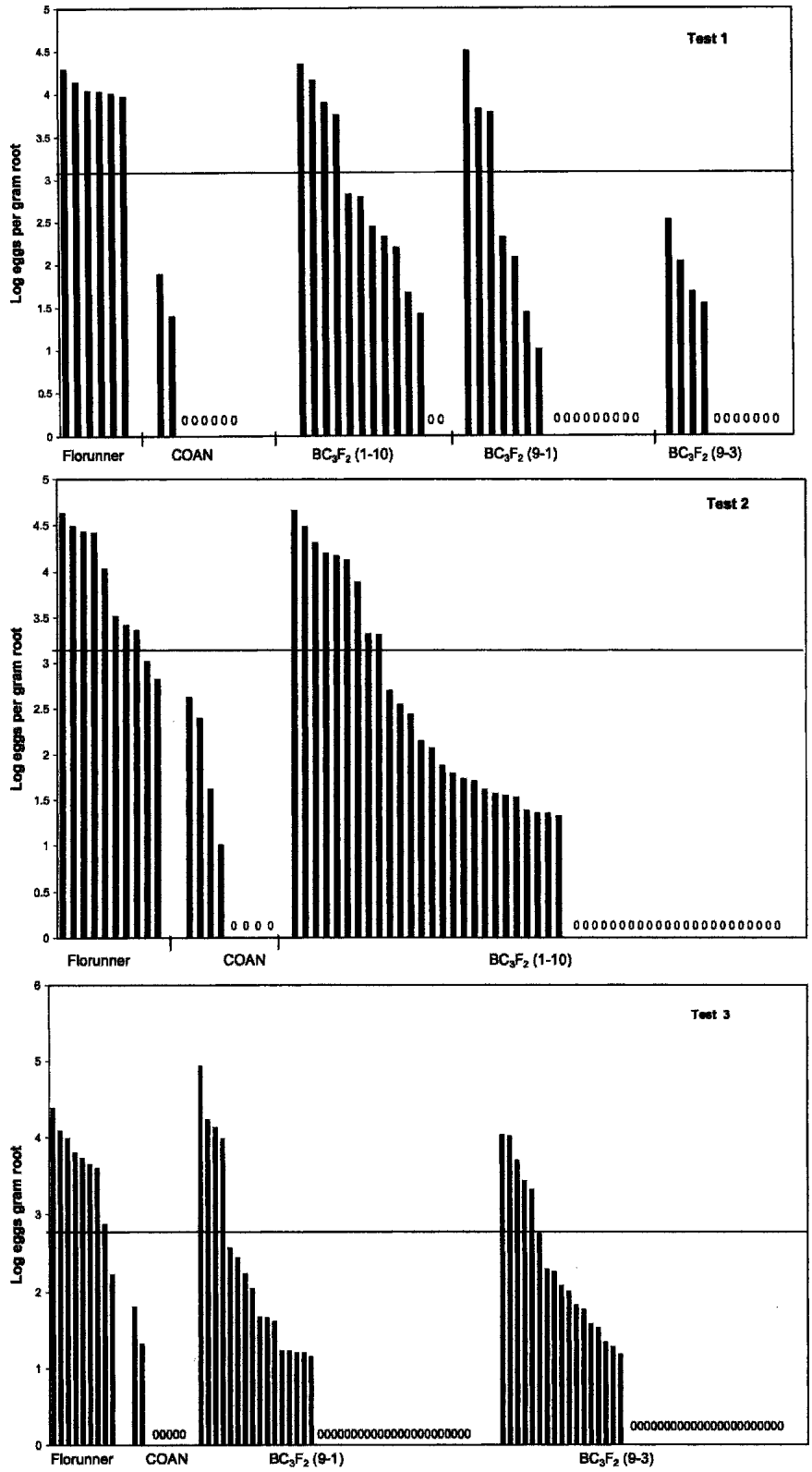

FIG. 3. Egg production per gram root of *Meloidogyne arenaria* on 215 individual F_2 plants from the BC_3F_2. Data are given for three tests. The horizontal lines represent 10% of the mean numbers of eggs per gram root produced on the susceptible cultivar Florunner. COAN and TxAG-6 are resistant controls. Individuals with no eggs detected are marked with 0.

TxAG-6 fit the expected values for one dominant gene and one recessive gene. These results were supported by segregation patterns in F_2 progeny from three BC_3 breeding populations. The hypothesis that resistance in TxAG-6 and the BC_3 lines is conditioned by one dominant gene and one recessive gene was further tested by

TABLE 3. Chi-square analysis of segregation for resistance in the F_3 generation from susceptible BC_3F_2 individuals and susceptible F_2 individuals from the cross *Arachis hypogaea* cv. Florunner × TxAG-6.

Susceptible F_2 individual	Observed		Expected		χ^2
	R	S	R	S	
	BC_3F_3				
1-10-14	3	20	5.7	17.3	1.75
1-10-5	2	4	1.5	4.5	0.22
	A. hypogaea × TxAG-6				
4-7	2	33	8.8	26.2	6.94*
4-8	4	42	11.5	34.5	6.52*

* Deviates from the expected value for a 1:3 R:S segregation ratio ($P = 0.05$); critical value is 3.84.

evaluating the F_3 generation from F_2 individuals that were identified as susceptible. If resistance is conditioned by both a dominant and recessive gene, then susceptible individuals in the F_2 generation have a 67% chance of being in the heterozygous condition for the recessive resistance gene. The F_3 generation from individuals heterozygous for the recessive gene would segregate in a 1:3 (R:S) ratio. The susceptible BC_3F_2 individuals 1-10-14 and 1-10-5, derived from 1-10, had F_3 progeny that fit the expected segregation pattern. The susceptible F_2 individuals 4-7 and 4-8, derived from *A. hypogaea* Florunner × TxAG-6, segregated in a ratio that was significantly different from the expected ratio. However, resistant individuals were recovered from the susceptible F_2 parents, suggesting that at least one recessive gene was present. It is unlikely that these individuals appeared resistant due an error in the evaluation of the resistant phenotype because no such escapes were observed among the susceptible controls in these tests. The level of reproduction of individuals possessing the recessive resistance gene appears to be similar to that of the previously identified dominant gene, which is near immunity in greenhouse experiments (Burow et al., 1996; Choi et al., 1999).

The molecular markers, R2430E and S1018E, flanking the previously identified dominant resistance gene (Church, 2002) were intended to be used to identify individuals that possessed additional resistance genes. Initial results from the molecular markers, greenhouse, and microplot experiments suggested individuals 9-1, 9-3, and 1-10 from BC_3F_1 were resistant to *M. arenaria* but lacked the resistance locus, indicated by the lack of locus R2430E. Further study revealed that 9-1 and 9-3 possessed the flanking S1018E locus, indicating the resistance locus was still present in those individuals. The individual, 1-10, lacked both flanking markers. The probability that an individual would lack the single dominant resistance gene found in COAN, if it lacked both loci, is 99.98% based on the results of linkage analysis (Church et al., 2001). Subsequent evaluation of the F_2 generation indicated that the dominant resistance gene was present. This hypothesis was further supported by the segregation ratios from the F_2 generation from the cross between *A. hypogaea* Florunner × TxAG-6. Considering 1-10 was the only plant of 420 BC_3F_1 individuals (data not shown) that lacked both flanking markers and was resistant, it is likely that a double crossover occurred in this individual. The double crossover resulted in the loss of the markers without loss of the gene. In this case the molecular markers were not directly useful in identifying additional resistance genes.

The identification of this additional recessive gene for resistance to *M. arenaria* may allow development of more durable resistance. The development of germplasm and cultivars with the dominant and recessive resistance genes will reduce the probability of virulent

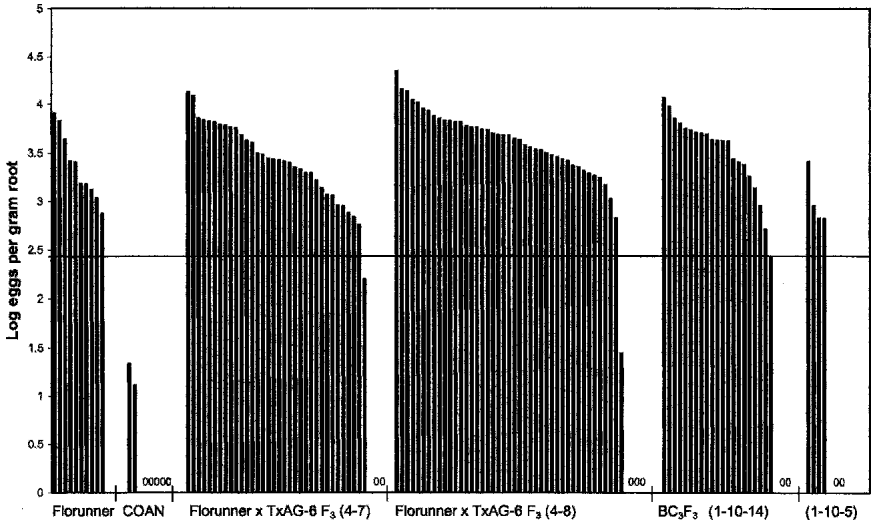

FIG. 4. Egg production per gram root of *Meloidogyne arenaria* on 110 individual F_3 plants from the cross between *Arachis hypogaea* cv. Florunner × TxAG-6 and the BC_3F_2. The horizontal lines represent 10% of the mean numbers of eggs per gram root produced on the susceptible cultivar Florunner. COAN and TxAG-6 are resistant controls. Individuals with no eggs detected are marked with 0.

populations developing and therefore provide durable resistance. Additional research is needed to identify which wild *Arachis* spp. contributed this recessive resistance gene to TxAG-6, develop markers linked to this locus, and determine the resistance mechanism conditioned by this gene.

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