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BREEDING AND GENETICS

Inheritance of Root-knot Nematode Resistance in M-315 RNR and M78-RNR Cotton

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ABSTRACT

The southern root-knot nematode (RKN) [*Meloidogyne incognita* (Kofoid & White)] is a serious pest of cotton (*Gossypium hirsutum* L.) with detrimental effects being most pronounced on sandy soils that are also infested with the Fusarium wilt pathogen. Varietal resistance is an effective method of managing the RKN/Fusarium wilt complex. In 1970, a high level of RKN resistance was developed in the germplasm line Auburn 623 RNR, but no commercial cultivar has been developed with this near-immunity level of resistance. The objective of this study was to evaluate the mode of inheritance of RKN resistance in M-315 RNR (M-315), a germplasm line with the Auburn 623 RNR source of resistance, and in M78-RNR, a day-neutral version of the race stock line T78. These lines were crossed with M8, an RKN-susceptible cotton line, and with each other. The parental, F_1, F_2, and backcross generations of these crosses were evaluated in the greenhouse for RKN reproduction 40 d after planting in a Wickham sandy loam soil that had been infested with either 5,000 or 10,000 RKN eggs per pot. The minimum number of genes conditioning resistance in M-315 and M78-RNR was estimated at two and one, respectively. Mendelian analyses indicated that a two gene, one dominant (Mi_1) and one additive (Mi_2), model fit the data for M-315. The data from crosses with M78-RNR indicated that it had only the dominant Mi_1 gene. These data indicate that the Auburn 623 RNR source of RKN resistance should be easily transferable to commercial cultivars.

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The southern root-knot nematode (RKN) is a serious pest of cotton, primarily on lighter textured soils. Root-knot nematode infection impairs cotton root function, which severely limits plant growth (O'Bannon and Reynolds, 1965), and predisposes the plant to Fusarium wilt infection (Martin et al., 1956). The RKN/Fusarium wilt complex is more debilitating than either disease alone (Starr et al., 1989); however, a high level of resistance to RKN is reported to also convey field resistance to the RKN/Fusarium wilt complex (Shepherd; 1982a).

Host plant resistance is an effective and environmentally benign method for controlling RKN in cotton. Even though highly resistant germplasm lines are available, cotton cultivars with high levels of resistant to RKN are not available (Jenkins et al., 1993). Three commercially available cultivars ST LA887, PM1560, and Acala NemX have a moderate level of resistance. The highly resistant breeding line Auburn 623 RNR (A623) was derived from transgressive segregation in the cross 'Clevewilt 6-8' (SA 235) with 'Mexico Wild' (TX 2156) (Shepherd, 1974). In greenhouse studies, fewer RKN eggs were recovered from A623 RNR 40 d after inoculation than were initially added to the pots (Shepherd, 1979). Preliminary reports indicated the inheritance of the A623 RNR source of RKN resistance was multigenic and partially dominant (Shepherd, 1974). In working with two different lines derived from the A623 RNR source, McPherson (1993) and Zhou (1999) suggested that the high level of RKN resistance in these lines was controlled by only two major genes. McPherson et al. (1995) postulated that the RKN resistance in A623 RNR was simply inherited and that it received one resistance gene from each parent, Clevewilt 6-8 and Mexico Wild. In a study designed to identify molecular markers linked with RKN resistance, Bezawada et al. (2003) determined that the F_2 from the cross of 'Clevewilt 6-1' and 'Stoneville 213' fit a recessive one-gene model for resistance. Similarly, Zhou et al. (1999) indicated that a single gene controlled the RKN resistance in Acala NemX, and depending on how the resistance classes were

defined, the data fit either a recessive or an additive one-gene model. These results support the premise of simple inheritance for RKN resistance.

The resistance of A623 RNR has been transferred to lines with progressively better agronomic characters, including Auburn 634 RNR and M-315. By backcrossing A623 to Auburn 56, Auburn 634 RNR was developed, which is comparable in yield to Auburn 56 (Shepherd, 1982b). Germplasm line M-315 was developed by backcrossing Auburn 634 RNR to 'Deltapine 61' and was released along with eight other lines with high RKN resistance and improved agronomic traits (Shepherd et al., 1989). In a search for additional sources of RKN resistance, Shepherd (1983) evaluated 471 primitive accessions of cotton and identified 18 with moderate levels of resistance. Twelve of these accessions have been released as day-neutral converted germplasm lines, including M78-RNR (Shepherd et al., 1988). The objective of this research was to evaluate the mode of inheritance of RKN resistance in M-315 and M78-RNR.

MATERIALS AND METHODS

The RKN population used in this study originated from a single egg mass isolated from a cotton plant grown at the E.V. Smith Research Center at Tallassee, AL. The RKN population was subsequently maintained continuously in the greenhouse on M8 (RKN-susceptible) cotton. This nematode isolate was confirmed as race 3 of *M. incognita* by the differential host test (McPherson, 1993).

The plants were grown in 8.9 x 7.6 cm (dia x h), plastic pots that had been recessed in the soil on the greenhouse benches and filled with dry, screened, methyl bromide-fumigated, Wickham sandy loam soil (fine- loamy, mixed, semiactive, thermic Typic Hapudults). The soil was wetted and a 2-ml aliquot of 5,000 or 10,000 RKN eggs was pipetted into a cylindrical 1 x 2 cm (dia x h) hole in the soil of each pot. Following inoculation, the pots were covered for 7 d with sequential layers of clear plastic, brown paper and aluminum foil to promote the hatching of RKN eggs and the dispersal of juveniles through the soil. The cover was removed after 7 d and each pot was planted with a single seed. Five consecutive pots in a row were planted from the same seed source and were treated as an experimental unit or plot. The experiments were harvested 40 d after each planting date. The top of each plant was excised 10 cm above the soil line and the soil was gently rinsed from the five root systems of a plot with a high volume, low pressure spray of water.

Root knot nematode resistance was based on nematode reproduction as determined by either the number of egg masses per plant or by the number of eggs per plant. To count egg masses, the roots were placed in Phloxine B stain (50 mg in 700 ml water) for at least 15 min. Using a 7X stereoscope and a grid, the number of RKN egg masses on the root system of each plant was counted. For number of eggs per plant, the gelatinous matrix of the egg masses was dissolved with dilute NaOCl. The five root systems of a plot were shaken for 4 min in a 500-ml sealed container with 200 ml of 1.05% NaOCl and the number of eggs per plant was subsequently estimated by dilution counting.

Experiment 1. The RKN susceptible line M8 was used as a female in crosses with M-315 and M78-RNR. To generate the populations required for a generation mean analysis, the F_1 of each cross was self-pollinated and backcrossed once to each parent. The parental, F_1, F_2, and backcross generations of these crosses were evaluated for RKN resistance, as measured by the number of egg masses per plant in a greenhouse experiment planted on 21 April, 19 May, and 8 June in 1992. The experiment was designed as a split-plot treatment arrangement in a randomized complete block with two replications for each date. Whole-plot treatments were the two crosses with M8 and the subplots were the six generations. At 7 d prior to each planting date, each pot was inoculated with approximately 5,000 RKN eggs. The number of pots planted to each generation per replication varied as follows: 5 pots of M8, 10 pots each of the resistant parent and the F_1, 30 pots of the F_2, and 15 pots each of the two backcross generations. Although the plants for both crosses were grown concurrently for each date, the data were analyzed separately by cross.

Experiment 2. To determine if the genes for resistance in M78-RNR were allelic to those in M-315, these two lines were crossed, and the F_1 was backcrossed to M78-RNR (BCP_2). The parental, F_1, F_2, and BCP_2 generations were evaluated for RKN resistance (egg masses per plant) in a greenhouse experiment planted on 25 April, 12 June, and 1 July in 1991. The experiment was designed as a randomized complete block with two replications. At 7 d prior to each planting date, each pot was inoculated

with approximately 10,000 RKN eggs. The number of pots planted to each generation per replication varied as follows: 5 pots each of the parents and the F_1, 40 pots of the F_2, and 20 pots of the BCP_2.

Experiment 3. All 28 possible single crosses were made among M8, M-315, M19-RNR, M25-RNR, M75-RNR, M78-RNR, M188-RNR and M487-RNR. The F_1 generation of these crosses was evaluated for RKN resistance (eggs per plant) in greenhouse experiments planted on 20 August 1991 and 30 March 1992. Each experiment was designed as a randomized complete block with six replications of five pots for each cross. At 7 d prior to each planting date, each pot was inoculated with approximately 10,000 RKN eggs. The combining abilities of these lines for RKN resistance were reported by McPherson et al. (1995), but data for crosses among M8, M-315, and M78-RNR that were not previously reported are presented.

Statistical analysis. The data were subjected to both quantitative and qualitative analyses. An analysis of variance and a generation mean analysis were performed on data from Experiments 1 and 2 according to Hayman (1958). The minimum number of genes (n_1 to n_4) controlling RKN resistance in these lines was estimated using parental means (P_1 and P_2) and estimates for the segregating genetic variance (s^2_s) in a formula described by Castle (1921):

$$n_x = (P_1 - P_2)^2 * (8s^2_{sx})^{-1} \quad (1)$$

Lande (1981) presented four methods to calculate the segregating genetic variance component (s^2_s) of the Castle-Wright formula as follows:

$$s^2_{S1} = s^2_{F2} - s^2_{F1} \quad (2)$$

$$s^2_{S2} = s^2_{F2} - (0.5s^2_{F1} + 0.25s^2_{P1} + 0.25s^2_{P2}) \quad (3)$$

$$s^2_{S3} = 2s^2_{F2} - s^2_{BCP1} - s^2_{BCP2} \quad (4)$$

$$s^2_{S4} = (s^2_{BCP1} + s^2_{BCP2}) - (s^2_{F1} + 0.5s^2_{P1} + 0.5s^2_{P2}). \quad (5)$$

Subsequently, Cockerham (1986) corrected the parental means by their standard errors and combined the formulae for genetic variance into one by using least squares to give another estimate for gene number (M):

$$M = [(P_1 - P_2)^2 - (s^2_{P1}N^{-1} + s^2_{P2}N^{-1})] * (8s^2_{s5})^{-1} \quad (6)$$

where N = number of plants and

$$s^2_{S5} = 0.2(4s^2_{F2} + s^2_{BCP1} + s^2_{BCP2}) - 0.4(s^2_{P1} + s^2_{P2} + s^2_{F1}). \quad (7)$$

The minimum number of genes for resistance in these lines was estimated using all five of these formulae.

According to Lande (1981), the validity of gene number estimates depends upon the choice of a scale that renders 1) the generation means additive, 2) the parental and F_1 variances uniform with the extra variance of the backcrosses being half that of the F_2, and 3) the standard errors normally distributed. Lande (1981) demonstrated that a modified logarithmic transformation successfully transformed several data sets to satisfy these assumptions for estimating the number of genes. Using the methods of Wright (1968), the data for each plant were transformed by log10 (egg mass + 4.6) before the minimum number of genes was estimated.

Since the estimated number of genes was low, the data from each generation were subjected to a Mendelian analysis using a chi-square goodness-of-fit test in addition to the intended generation mean analysis. The frequency distributions of egg masses per plant for the parental and F_1 generations were used to define the intervals for the resistance classes.

RESULTS

Experiment 1. The analysis of variance indicated that neither planting dates nor the interaction of dates with generations was significant for either cross, but the generation term was significant for both crosses. The data were combined over planting dates for further analysis. A generation mean analysis (McPherson, 1993) indicated that an additive/dominance model was adequate to explain the data, and that both the additive and dominance parameter estimates were significant for both crosses (data not shown). The dominance effect for M-315 was 66% as large as its additive effect, and the dominance effect for M78-RNR was 87% as large as its additive effect, indicating that the RKN resistance of these lines was at least partially dominant to susceptibility.

The minimum number of genes for resistance in M-315 was consistently estimated to be two, while the number of genes in M78-RNR was estimated to be one (Table 1). Since the number of genes controlling RKN resistance in these lines was estimated to be only one or two, a Mendelian analysis was performed. Luedders (1989) noted that the division of a plant population with a continuously varying response to a pathogen into discrete classes was complicated by the number and dominance of resistance genes in the host and by the frequency of virulence genes in the pathogen. Our approach to this problem was to use the distributions for non-segregating

(parental and F_1) populations to establish resistance class intervals.

Table 1. Estimates for the minimum number of genes controlling resistance to *Meloidogyne incognita* in M-315 RNR and M78-RNR cotton

Estimate [z]	Number of genes	
	M-315	M78-RNR
n_1	2.2 ± 0.4	1.1 ± 0.5
n_1	2.0 ± 0.3	0.8 ± 0.2
n_3	2.0 ± 0.7	0.5 ± 0.2
n_4	2.1 ± 0.5	1.4 ± 0.9
M	2.0 ± 0.8	0.7 ± 0.4

[z] The minimum number of genes (n_1 to n_4) estimated using the four formulae developed by Lande (1981), and gene number (M) estimated using the formula of Cockerham (1986).

The frequency distributions for M-315, M8, and M-315 x M8 F_1 suggested four discrete phenotypic classes for the segregating generations as follows: highly resistant (HR), resistant (R), moderately resistant (MR) and susceptible (S). The range for number of egg masses in each class was defined by the distribution of egg masses per plant on M-315 (HR), the F_1 (R), and M8 (S). Although there was no generation to actually delineate the MR class, the gap in counts of egg masses between the F_1 and M8 was defined as MR for this research. The upper limit of the R class was very close to the mean of the M-315 x M8 F_1 generation plus one standard deviation. Since the frequency distribution was approximately equal for the R and MR classes in M78-RNR (data not shown), this lower level of resistance was defined as MR.

A genetic model that gave a good fit to the data for the cross with M-315 was one dominant gene and one additive gene that combined in an additive fashion. The BCP_1 [(M-315 x M8) x M-315], the BCP_2 [(M-315 x M8) x M8], and the F_2 generations fit expected phenotypic ratios of 1HR:1R, 1R:1MR:2S, and 3HR:6R:4MR:3S, respectively (Table 2). These ratios were derived by combining the genotypes that were expected to have the same phenotype using the putative one dominant and one additive gene model (Table 4). The dominant gene was designated as Mi_1 and the additive gene as Mi_2.

Since the frequency distributions for both M78-RNR and the F_1 of this line with M8 coincided, a one dominant gene model was postulated for M78-RNR. The F_2 and the BCP_2 [(M78-RNR x M8) x M8] generations fit 3MR:1S and 1MR:1S ratios, respectively (Table 3).

Table 2. Observed phenotypic distributions for six generations of M-315 x M8 for reaction to *Meloidogyne incognita*

Generation	Mean	Variance	Reaction (egg masses/plant) [z]				Ratio	X^2	P
			HR (0-15)	R (16-60)	MR (61-140)	S (>140)			
M-315	4	16	53	1	0	0	NA	NA	
F1 x M-315	18	186	41	40	2	0	1:1	0.01	0.90
F1	40	406	3	43	6	0	NA	NA	
F2	73	5320	26	71	39	27	3:6:4:3	2.77	0.25
F1 x M8	125	7699	4	19	26	32	1:1:2	3.76	0.10
M8	232	6404	0	2	0	26	NA	NA	

[z] HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

Table 3. Observed phenotypic distributions for six generations of M78-RNR x M8 for reaction to *Meloidogyne incognita*

Generation	Mean	Variance	Reaction (egg masses/plant) [z]		Ratio	X^2	P
			MR (0-140)	S (>140)			
M78-RNR	60	920	57	2	NA	NA	
F_1 x M78-RNR	46	1162	87	2	NA	NA	
F_1	64	1514	45	4	NA	NA	
F_2	108	4793	119	51	3:1	2.27	0.10
F_1xM8	147	3978	41	49	1:1	0.36	0.50
M8	233	6873	4	24	NA	NA	

[z] MR = moderately resistant; S = susceptible.

Table 4. Hypothesized genotypes, resistance ratings, and class frequencies for six generations of the cross M-315 x M8 using a two-gene model for RKN resistance

Generation	Genotypes	Egg masses	Rating [z]	Frequency
M-315	$Mi_1Mi_1Mi_2Mi_2$	0-15	HR	NA
F_1 x M-315	$Mi_1\_\_\ Mi_2Mi_2$	0-15	HR	0.5
	$Mi_1\_\_\ Mi_2mi_2$	16-60	R	0.5
F1	$Mi_1mi_1Mi_2mi_2$	16-60	R	NA
F 2	$Mi_1\_\_\ Mi_2Mi_2$	0-15	HR	0.1875
	$Mi_1\_\_\ Mi_2mi_2$	16-60	R	0.375
	$Mi_1\_\_\ mi_2mi_2 + mi_1mi_1Mi_2Mi_2$	61-140	MR	0.25
	$mi_1mi_1Mi_2mi_2 + mi_1mi_1mi_2mi_2$	>140	S	0.1875
F_1 x M8	$Mi_1mi_1Mi_2mi_2$	16-60	R	0.25
	$Mi_1mi_1mi_2mi_2$	61-140	MR	0.25
	$mi_1mi_1Mi_2mi_2 + mi_1mi_1mi_2mi_2$	>140	S	0.5
M8	$mi_1mi_1mi_2mi_2$	>140	S	NA

[z] HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

Experiment 2. For the allele test between M-315 and M78-RNR, the generation term was significant, but neither the date nor the date by generation interaction was significant. As with the data for Experiment 1, the egg mass data for this cross were combined over dates, the frequency distributions were determined, and discrete classes were partitioned. Since the number of eggs in the inoculum for this experiment was twice that for Experiment 1, the class intervals were significantly different from those in Experiment 1. The level of egg mass production for M78-RNR in Experiment 1 was only 51% of the average between M-315 and M8, but it was 79% of this average in Experiment 2. The data fit a model of one additive gene for RKN resistance segregating in the cross between M-315 and M78-RNR (Table 5). In support of this hypothesis, the mean number of egg masses on the F_1 and F_2 of this cross were intermediate to the two parents, and the backcross to M78-RNR was intermediate to the F_1 and M78-RNR.

Experiment 3. The analysis of variance for the combining ability study indicated that the interaction of dates with crosses was significant. The interaction was due to higher egg production on the second planting date for crosses with less resistant parents. Even though RKN reproduction was very different on M-315, M78-RNR, and M8 in Experiment 1 (Tables 2 and 3) and in Experiment 2 (Table 5), the number of eggs recovered from M315 x M78-RNR and M315 x M8 was nearly identical for both planting dates of Experiment 3 (Fig. 1). The dominant Mi_1 gene from M78-RNR did not increase the resistance of the M315 x M78-RNR cross beyond that of the M315 x M8 cross, because M-315 and M78-RNR both contributed the dominant Mi_1 gene, but neither M78-RNR nor M8 had the additive Mi_2 gene to compliment the one received from M-315. The M78-RNR x M8 cross was less resistant than the M315 x M8 cross because it carried only the dominant Mi_1 gene.

Table 5. Observed phenotypic distributions for five generations of M-315 x M78-RNR and M8 for reaction to *Meloidogyne incognita*

Generation	Mean	Variance	Reaction (egg masses/plant) [z]				Ratio	X^2	*P*
			HR (0-15)	R (16-65)	MR (66-175)	S (>175)			
M-315	8	52	28	2	0	0	NA	NA	
F_1	55	499	2	18	5	0	NA	NA	
F_2	54	2001	59	105	60	4	1:2:1	1.74	0.25
F_1 x M78-RNR	71	1620	6	57	47	1	1:1	2.03	0.10
M78-RNR	122	5174	0	6	13	6	NA	NA	
M8 (check)	299	8054	0	0	1	25	NA	NA	

[z] HR = highly resistant; R = resistant; MR = moderately resistant; S = susceptible.

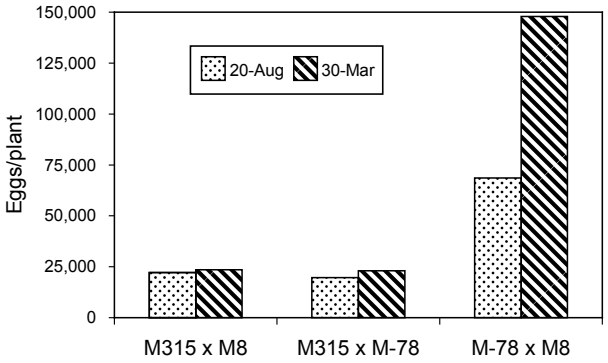

Fig. 1. Average number of *Meloidogyne incognita* eggs per plant recovered from the M-315 x M78-RNR, M-315 x M8, and M78-RNR x M8 crosses on two planting dates.

DISCUSSION

The observed data provided a good fit to expected F_2 and backcross ratios using classes based on the distributions of the non-segregating generations. A two-gene (one dominant, Mi_1, and one additive, Mi_2) model fit the data for the M-315 x M8 cross, a one-gene (dominant) model fit the data for the M78-RNR x M8 cross, and a one-gene (additive) model fit the data for the M-315 x M78-RNR cross. The gene for resistance in M78-RNR was apparently allelic to the Mi_1 gene of M-315, but it did not carry the additive Mi_2 gene.

These models are supported by data on RKN development presented by Jenkins, et al. (1995). The infectious second-stage juveniles (J2) penetrated the roots of susceptible M8, moderately resistant M78-RNR, and highly resistant M-315 to similar levels, and the degree of root galling was similar in all three cotton lines at 6 days after inoculation (DAI). The number of RKN in M-315 rapidly decreased at 8 DAI and remained relatively constant until 24 DAI when it started decreasing again. The primary difference between M-315 and M8 at 8 DAI was in the number of J2 larvae that developed into J3 and J4 larvae. The number of RKN and the stages of RKN development in M78-RNR were very similar to M8 until 24 DAI when the number of surviving RKN started declining. Jenkins et al (1995) proposed that one gene was actively expressed in both M-315 and M78-RNR at 24 DAI and that a second gene was expressed only in M-315 at 6 DAI. This second gene in M-315 may be the MIC-3 gene that Zhang et al. (2002) cloned from a primer developed from a 14-kDa protein that was induced at 8-10 DAI in M-249 in response to RKN infection. Line M-249 is a highly RKN-resistant line with the A623 source of resistance (Shepherd et al., 1989).

To identify criteria to effectively breed for the high level of resistance in A623, Shepherd (1979) screened 1,110 F_2 plants from a cross of A623 with a susceptible parent, and progeny tested the F_2 plants with less than 16 RKN egg masses per plant. The number of egg masses on A623 was not stated, but there were 255 RKN eggs in egg masses on A623 that yielded a total of 1,000 eggs per plant. If A623 had up to 6 egg masses per plant, then 21% of the F_2 plants were as resistant as A623. This is very close to the 18.75% (3/16) predicted by the two-gene model in this study with M-315; however, when the resistant F_2 plants were tested as F_3 progeny, only 1.5% of the F_2 plants produced progeny that were all highly resistant and putatively homozygous for resistance. The observance of only 1.5% true-breeding, highly resistant F2 plants indicated that at least three genes for RKN resistance were responsible for the very high level of resistance in A623. Shepherd (1983) reported that T78, the photoperiodic recurrent parent of M78-RNR, was nearly as resistant as Auburn 634 RNR, but in the present study M78-RNR had a much lower level of resistance. This indicates that one of the putative resistance genes may have been lost during the process of introgressing alleles for day-neutrality into M78-RNR.

According to the proposed two-gene model, only one Mi_1 allele is needed for the M78-RNR level of resistance, and the addition of Mi_2 alleles increases resistance in an additive fashion. The observed levels of RKN resistance for the three parents and the three crosses among them were as follows: M-315 (Mi_1Mi_1 Mi_2Mi_2) > M315 x M78 = M315 x M8 (Mi_1_Mi_2mi_2) > M78-RNR = M78 RNR x M8 (Mi_1_mi_2mi_2) > M8 (mi_1mi_1 mi_2mi_2).

The reported one-gene models for resistance in Clevewilt 6-1 (Bezewada et al., 2003) and Acala NemX (Zhou et al., 1999) may be the same as the Mi_2 gene. When homozygous, Mi_2 may confer a low level of RKN resistance similar to that observed for M78-RNR of the present study; however, when heterozygous in the absence of a dominant allele at the Mi_1 locus, the phenotype may be practically indistinguishable from a RKN susceptible line. Since the level of RKN resistance conferred by Mi_2 alleles is additive in the presence of a dominant allele at the Mi_1 locus, the gene action for Mi_2 is additive. The M78-RNR x Acala NemX cross could be used

to confirm the two-gene model. One would expect the F_1 to be more resistant than either parent, and the self-pollinated F_2 to yield 1/16 pure-breeding, highly resistant progeny.

The two-gene model suggests that the high level of RKN resistance in M-315 and other lines derived from A623 may be transferred more easily to better agronomic types than was initially thought. Development of closely linked molecular markers for these two genes would greatly facilitate the development of RKN resistant cultivars by commercial companies, because it would preclude the necessity of using greenhouse screening to identify plants carrying the resistance genes.

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