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I. Identification of AFLP Markers Linked to the *N* Gene for Root-knot Nematode Resistance in Pepper

Root-knot nematodes, *Meloidogyne* spp., are major limiting factors to pepper production in the Southern states and California. Fumigation with methyl bromide is the primary control method in bell pepper and this practice accounts for 12% of all methyl bromide used for pre-plant treatments in the U.S. The proposed ban of methyl bromide in the U.S. in 2005, and the loss of many other nematicides due to environmental concerns and prohibitive registration costs, has focused major interest on host resistance to nematodes, a very difficult breeding objective, as a viable management alternative. Scientists at the U.S. Vegetable Laboratory developed the first two bell pepper cultivars with root-knot nematode resistance conferred by the *N* gene; the cultivars Carolina Wonder and Charleston Belle were released in 1997. Presently, these two open-pollinated root-knot nematode resistant cultivars are used extensively in commercial breeding as a source of resistance for bell pepper hybrids. However, current breeding methods rely on costly and time-consuming greenhouse bioassays of breeding lines for reaction to root-knot nematodes (length of test is typically 12 to 15 weeks). The development of high-throughput molecular markers linked to *N* will allow breeders to use marker-assisted selection (MAS) to rapidly develop root-knot nematode resistant genotypes.

MATERIALS and METHODS

The purpose of this study is to identify genetic markers for the *N* gene in pepper that can be used as tools for isolating this gene and developing root-knot nematode resistant commercial pepper cultivars.

Plant populations: Pepper genotypes used in these experiments were ‘Carolina Wonder’ (*NN*), ‘Mississippi Nemaheart’ (*NN*), ‘Yolo Wonder B’ (*nn*), and BC6-F2 Populations I and II (‘Mississippi Nemaheart’ x ‘Yolo Wonder B’) segregating for the *N* gene. ‘Carolina Wonder’ is a bell pepper cultivar with a high level of resistance to the southern root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood], which was developed and released by the U.S. Vegetable Laboratory, Agricultural Research Service of the U.S. Department of Agriculture. ‘Carolina Wonder’ is the product of a conventional recurrent backcrossing program to transfer the dominant *N* gene for root-knot nematode resistance from ‘Mississippi Nemaheart’, a pimiento pepper, into ‘Yolo Wonder B’. ‘Carolina Wonder’ originated from bulked F3 populations that were derived after the sixth backcross.

Bioassay #1 for root-knot nematode resistance: Seeds of ‘Mississippi Nemaheart’, ‘Yolo Wonder B’, ‘Carolina Wonder’, and the BC6-F2 Population I were sown in flats containing

Metro-Mix® in the greenhouse. After 21 days, the seedlings were transplanted into a raised 4.0 x 1.8 x 0.25-m greenhouse bench containing a steam-pasteurized medium of 2 fine washed river sand: 1 sandy loam soil (vol/vol). At transplanting, 5 mL tap water containing approximately 3000 eggs of *M. incognita* race 3 were pipetted around the base of each plant. Twelve weeks after inoculation with *M. incognita*, the root system of each plant was removed from the growing medium, washed, and rated for severity of root galling and egg mass production using a 1 to 5 scale in which: 1 = 0% to 3% root system galled or covered with egg masses, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 80%, and 5 = > 80% root system galled or covered with egg masses. Root gall and egg mass indices <3 are considered resistant reactions. Numbers of plants evaluated in this bioassay: ‘Mississippi Nemaheart’, 40; ‘Yolo Wonder B’, 44; ‘Carolina Wonder’, 40; and BC6-F2 Population I, 97.

Bioassay #2 for root-knot nematode resistance: The study was conducted as previously described except that seedlings were transplanted from flats containing Metro-Mix® into 15-cm diameter clay pots filled with a steam-pasteurized medium of 2 fine washed river sand: 1 sandy loam soil (vol/vol). Ten days after transplanting, 5 mL tap water containing approximately 3300 eggs of *M. incognita* race 3 were pipetted around the base of each plant. Twelve weeks after inoculation with *M. incognita*, the root system of each plant was removed from the growing medium, washed, and rated for severity of root galling and egg mass production using the 1 to 5 scales described above. Numbers of plants evaluated in this bioassay: ‘Mississippi Nemaheart’, 22; ‘Yolo Wonder B’, 19; ‘Carolina Wonder’, 23; and BC6-F2 Population II, 73.

DNA extractions: Young pepper leaves were collected from the F2 and control cultivars in Bioassays #1 and #2 and placed directly in the –80°C freezer. Approx. 5 g leaves were removed from the freezer and ground to a complete powder using 2 g sand (white quartz ; -50 +70 mesh) and three rounds of liquid N. DNA was isolated using a modified CTAB procedure described by Levi and Thomas (2004).

AFLP analysis: DNA analysis to find polymorphic markers among the resistant and susceptible cultivars were performed using AFLP procedures (Vos et al., 1995). Pepper genomic DNA (0.5ug) from ‘Yolo Wonder B’, ‘Mississippi Nemaheart’, and ‘Carolina Wonder’ was digested with EcoRI and MseI restriction enzymes and pre-selective amplifications of genomic DNA were performed using a commercially available kit (Plant Mapping Kit - Regular Plant Genome, Applied Biosystems; Foster City, CA) according to manufacturer’s protocol. Sixty-four EcoRI - MseI primer pairs were used in selective amplification. All reactions were carried out using MJ Research PTC-200 thermocyclers. Selective reactions were electrophoresed at 3,000 V on a 4.25% denaturing polyacrylamide gel using a Perkin Elmer ABI-373 or ABI-377 Sequencer, or a Beckman Coulter CEQ 8000 Capillary Sequencer according to manufacturer’s protocol. Fragment sizes were determined using Genescan and Genotyper software (Applied Biosystems) or CEQ 8000 Genetics Analysis System Fragments Software (Beckman Coulter).

RESULTS and DISCUSSION

Inheritance of resistance: Root-knot nematode bioassay #1. Generally, the parental cultivars reacted as expected. ‘Mississippi Nemaheart’ was resistant; the mean gall index was 1.8 (Fig. 1). However, four of 39 plants were considered susceptible with a gall index of 3.0. In previous

experiments we have noted an occasional plant of ‘Mississippi Nemaheart’ in the intermediate to susceptible range. It may be possible that the line is segregating for root-knot nematode resistance. ‘Yolo Wonder B’ was susceptible; the mean gall index was 4.2. ‘Carolina Wonder’ (near-isogenic to ‘Yolo Wonder B’) exhibited high resistance; the mean gall index was 1.3. The F2 population segregated 3 resistant : 1 susceptible ($\chi^2 = 0.89$, $P = 0.50$ to 0.25) which confirms previous reports by Fery and Dukes, that the *N* gene is a single dominant gene (Fig. 2).

Root-knot nematode bioassay #2. The parental cultivars reacted as expected. ‘Mississippi Nemaheart’ was resistant; the mean gall index was 1.2 (Fig. 3). ‘Yolo Wonder B’ was susceptible; the mean gall index was 4.2. ‘Carolina Wonder’ exhibited high resistance; the mean gall index was 1.1. The F2 population segregated 3 resistant : 1 susceptible ($\chi^2 = 1.17$, $P = 0.50$ to 0.25, Fig. 4) confirming results of the bioassay of Population I as described above.

AFLP analysis: The 64 primer pairs produced 234 polymorphic AFLP markers present in the cultivars Mississippi Nemaheart and Carolina Wonder, but absent in ‘Yolo Wonder B’ (Table 1). These markers are putatively linked to the *N* gene and will be re-confirmed as present in ‘Mississippi Nemaheart’ and ‘Carolina Wonder’, and absent in ‘Yolo Wonder B’. The best candidate markers will be tested against the F2 population.

Impact: The root-knot nematode bioassay studies confirmed the 3 resistant :1 susceptible segregation of the *N* gene for root-knot nematode resistance in F2 populations of ‘Mississippi Nemaheart’ x ‘Yolo Wonder B’. DNA was isolated from plants of the parental cultivars, the resistant near-isogenic cultivar, and F2 progeny. Two hundred thirty-four polymorphic AFLP markers were identified in ‘Mississippi Nemaheart’ (nematode resistant donor parent) and ‘Carolina Wonder’ (resistant line near-isogenic to ‘Yolo Wonder B’), but absent in ‘Yolo Wonder B’ (susceptible recurrent parent). Identification of molecular markers linked to the *N* gene will facilitate marker-assisted selection (MAS) and the isolation of the *N* resistance gene. The results of this research will develop host plant genetic resistance through breeding and DNA manipulation which includes: (a) development of molecular markers to identify and track resistance genes; (b) development of methods for detection and isolation of resistance genes; and (c) development of improved methods to identify, evaluate, and confirm resistance in large host plant populations.

II. Characterization of resistance to root-knot nematodes conditioned by the *N* and *Me* genes in pepper

The *N* gene and the *Me* genes have been reported to control resistance to root-knot nematodes in pepper, *Capsicum annuum* (Castagnone-Sereno et al. 2001; Hare, 1956; Hendy et al. 1985; Fery and Dukes, 1996). Hendy et al. (1985) observed five genes, designated *Me1* to *Me5* that control resistance to various *Meloidogyne* spp. Two of these genes, *Me1* and *Me3*, confer broad spectrum resistance to *M. incognita*, *M. arenaria*, and *M. javanica* (Hendy et al. 1985). Likewise, the *N* gene confers high resistance to *M. incognita*, *M. arenaria* races 1 and 2, and *M. javanica* (Thies and Fery, 2000). Although each gene system has been individually well characterized, resistance controlled by the two genetic systems has not been compared in a single study; e.g. there is no information about the relationship of the *N* and *Me* gene systems to each other and whether the *N* and *Me* genes are allelic.

MATERIALS and METHODS

The purpose of this study was twofold: (i) to characterize resistance to *Meloidogyne incognita* race 3 in pepper genotypes carrying the *N* or *Me* genes and (ii) to develop plant populations for allelism tests to elucidate whether the *N* and *Me* genes are at different loci.

Pepper genotypes: Twelve pepper (*Capsicum annuum*) genotypes that differ in presence or absence of the *N* and *Me* genes that confer resistance to *M. incognita* were evaluated in this test. ***N* gene:** ‘Carolina Cayenne’, ‘Charleston Belle’, and ‘Carolina Wonder’ are homozygous for the *N* gene (*NN*). PA-426 is homozygous dominant for the *N* gene or for an allele of the *N* gene. These four *C. annuum* genotypes were developed by scientists at the U.S. Vegetable Laboratory, USDA, ARS, Charleston, SC. ***Me* genes:** HDA 149, PM 687, PM 217, HDA 330 carry various allelic combinations of the *Me* genes. Seeds of these four *C. annuum* breeding lines were obtained from Dr. Allain Palloix (Centre de Recherche Agronomique d’Avignon, INRA, France). Two of the lines are doubled haploids (DH): HDA 149, developed through *in vitro* androgenesis from the F1 hybrid (PM 217 x ‘Yolo Wonder’), which contains only the *Me3* gene; and HDA 330, developed through *in vitro* androgenesis from the F1 hybrid (PM 687 x ‘Yolo Wonder’), which contains only the *Me1* gene. The remaining two lines are the progenitors of the DH lines: PM 687, an inbred line developed through selfing U.S. Plant Introduction (PI) 322719, which contains at least two genes, the *Me3* and *Me4* genes; and PM 217, an inbred line developed through selfing PI 201234, which contains at least two genes, the *Me1* and *Me2* genes. **Susceptible genotypes:** ‘California Wonder’, ‘Yolo Wonder B’, ‘Keystone Resistant Giant’, and PA-350 are *C. annuum* genotypes that are susceptible to *M. incognita*. These genotypes were included in the study as susceptible reference checks.

Inoculum. *Meloidogyne incognita* race 3 was cultured on ‘Rutgers’ tomato (*Lycopersicon esculentum* Mill.) and ‘Kentucky Wonder 191’ pole bean (*Phaseolus vulgaris* L.) in isolated soil benches in the greenhouse. Egg inocula were extracted from infected tomato and bean roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973).

Experimental design and data analysis. The experimental design was a randomized complete block with 5 replicates. Each replicate consisted of 5 plants per genotype. Nematode egg data

were $\log_{10}(x+1)$ transformed to normalize the data before analysis and back transformed data were reported. Data were analyzed using the GLM procedure of SAS for Windows System Version 6.12 (SAS Institute, Cary, N.C.) and means were separated using Duncan's multiple range test. Differences reported in the text were significant at the $P<0.05$ level.

Greenhouse test. Five 2-wk-old seedlings of each of 12 pepper genotypes were transplanted in a 10-cm square planting pattern in greenhouse benches containing steam-pasteurized 2 washed river sand : 1 sandy loam soil (vol : vol) on 3 March 2004. Five replicates of each genotype (5 plants per replicate, $n=25$) were planted. The pepper seedlings were inoculated on 12 March 2004 with 3,000 eggs of *M. incognita* race 3 in 3 mL tap water. On 4 May 2004, the roots were lifted and washed, and scored for root galling using a 1 to 5 scale where 1 = 0 to 3% of root system galled, 2 = 4 to 25%, 3 = 26 to 50%, 4 = 51 to 80%, and 5 = 81 to 100% of root system galled. Root systems were also rated for fibrous root mass using a 1 to 5 scale where 1 = large amount of fibrous roots (best); 3 = moderate amount of fibrous roots, and 5 = no fibrous roots present (worst). Nematode eggs were extracted from the entire fibrous root sample from each five-plant plot using the NaOCl method (Hussey and Barker, 1973). Three aliquots of each egg sample were counted using a stereomicroscope and the mean number of eggs per gram fresh root was reported.

RESULTS and DISCUSSION

All four genotypes (PA-426, 'Carolina Cayenne', 'Charleston Belle', and 'Carolina Wonder') that carry the *N* gene (or alleles of the *N* gene) exhibited high resistance to *M. incognita* (Table 2). No root galling or presence of visible egg masses was observed. Numbers of *M. incognita* eggs were very low (≤ 214 eggs per gram fresh root). The fibrous root index varied from 1.9 for 'Carolina Wonder' to 2.5 for PA-426. 'Carolina Wonder' had more fibrous roots than PA-426, PM 217, and all four of the susceptible check entries ($P<0.05$).

Two of the genotypes, HDA 149 (*Me3*) and PM 687 (*Me3* and *Me4*), that carry the *Me3* and/or *Me4* genes exhibited high resistance. No root galling or presence of visible egg masses was observed and numbers of *M. incognita* eggs were very low (≤ 96 eggs per gram fresh root) for both genotypes. PM 217, which carries *Me1* and *Me2*, exhibited moderately high resistance; minimal root galling was observed (gall index=1.8) and nematode reproduction was low (202 *M. incognita* eggs per gram fresh root). HDA 330, which carries the *Me1* gene, exhibited low resistance; moderate root galling was observed (gall index=2.5) and nematode reproduction was 8 to 31 \times greater than any of the other seven root-knot nematode resistant genotypes carrying either the *N* gene or *Me* gene(s). These results agree with a previous report that a collection of 22 isolates of *M. arenaria*, *M. incognita* and *M. javanica* were controlled by *Me1*, but one *M. arenaria* and two *M. incognita* isolates overcame resistance conferred by the *Me3* gene (Castagnone-Sereno et al. 2001). The fibrous root index varied from 2.1 for PM 687 to 2.4 for HDA 149.

All four of the susceptible check cultivars (Yolo Wonder B, PA-350, California Wonder, and Keystone Resistant Giant) exhibited susceptible reactions to *M. incognita*, as expected. Root galling was severe (gall indices ranged from 3.9 to 4.5) and nematode reproduction was high

(numbers of *M. incognita* eggs per gram fresh root ranged from 13,390 to 61,944). The fibrous root index varied from 2.6 for 'Keystone Resistant Giant' to 4.1 for PA-350. PA-350 had the least amount of fibrous roots of all entries evaluated in this test.

Allelism tests for *N x Me1* and *N x Me3*. F2 seed of 'Charleston Belle' x HDA 330, 'Carolina Wonder' x HDA 330, 'Charleston Belle' x HDA 149, and 'Carolina Wonder' x HDA 149 plus reciprocals is being produced.

Impact: The results of this test are interesting because there appears to be a range of reactions to *M. incognita* race 3 among the root-knot nematode resistant genotypes tested. All four genotypes carrying the *N* gene were highly resistant to *M. incognita*, but differences ($P < 0.05$) were observed in resistance conferred by different combinations of the *Me* genes. It appears that the *N* gene and the *Me3* gene both confer higher resistance than the *Me1* gene. However, the test must be repeated in order to confirm these results. In addition, allelism tests between the *N* and *Me* genes will be conducted in the near future in order to determine whether these two gene systems are different.

Personnel: AFLP assays were conducted by a permanent GS-8 Biological Science Technician funded by USDA, ARS. Graduate students and college students were hired to assist the GS-8 Technician with the root-knot nematode bioassays of F2 pepper populations, DNA extraction, characterization of *N* and *Me* germplasm in greenhouse, development of *N x Me* populations for allelism studies, and data analysis. A University of Charleston graduate student was hired to work May - August 2002 (40 hours per week), January 2003 – April 2004 (20 hours per week), and April – September 2004 (40 hours per week). A second University of Charleston graduate student was hired to work from 1 October 2002 through 5 April 2003 (20 hours per week). Two college student interns were hired June 2004 (40 hours per week); one intern worked 8 weeks and the second intern worked 12 weeks.

Literature Cited

- Castagnone-Sereno, P., M. Bongiovanni, and C. Djian-Caporalino. 2001. New data on the specificity of the root-knot nematode resistance genes *Me1* and *Me3*. *Plant Breeding* 120:429-433.
- Fery, R.L., and Dukes, P.D. 1996. The inheritance of resistance to the southern root-knot nematode in 'Carolina Hot' Cayenne pepper. *J. Amer. Soc. Hort. Sci.* 121:1024-1027.
- Hare, W.W. 1956. Resistance in pepper to *Meloidogyne incognita acrita*. *Phytopathology* 46:98-014.
- Hendy, H., E. Pochard, and A. Dalmasso. 1985. Inheritance of resistance to *Meloidogyne* Chitwood (Tylenchida) in 2 lines of *Capsicum annuum* L. Study of homozygous progenies obtained by androgenesis. *Agronomie* 5:93-100.
- Hussey, R. S. and Barker, K. R.. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Dis. Rep.* 57:1025-1028.
- Levi, A., Thomas, C.E., Newman, M., Reddy, O.U.K., Zhang, X., Xu, Y. ISSR and AFLP markers differ in watermelon cultivars with limited genetic diversity. 2004. *J. Amer. Soc. Hort. Sci.* 129(4) (*in press*).
- Thies, J. A., and R. L. Fery. 2000. Characterization of resistance conferred by the *N* gene to *Meloidogyne arenaria* races 1 and 2, *M. hapla*, and *M. javanica*. *J. Amer. Soc. Hort. Sci.* 125:71-75.
- Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Fritjers, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23:4407-4414.

Table 1. AFLP markers produced by EcoRI (E) and MseI (M) primer pairs present in ‘Mississippi Nemaheart’ and ‘Carolina Wonder’, and absent in ‘Yolo Wonder B’.^a

Primer pair	Marker size (base pairs)	
	Acrylamide gel procedure	Capillary gel procedure
E-AAC/ M-CAA		149,161,189,214,230,247
E-AAC/ M-CTA	78	
E-AAC/ M-CTC		153
E-AAC/ M-CTG		58
E-AAG/ M-CAA	114,190,371	
E-AAG/ M-CAC	95,255,273,460	
E-AAG/ M-CAT	96	
E-AAG/ M-CTC	319,352	
E-AAG/ M-CTG	87	
E-ACA/ M-CAA		57,76,104,112,120,145,149, 151,274
E-ACA/ M-CAC		5,84,93,96,141
E-ACA/ M-CAG		287
E-ACA /M-CTA		85,101,103,132,136,140,201, 214,217,219,221,228,260
E-ACA/ M-CTG	143	
E-ACA/ M-CTT	84,138,151,165	
E-ACC/ M-CAA		67,168,177
E-ACG/ M-CAA	69	
E-ACG/ M-CAC	112,160,266	
E-ACG/ M-CTC	62,83,95,98,100,113, 119,158,221,490	
E-ACT/ M-CAC	66,77	
E-ACT/ M-CAG		99
E-ACT/ M-CAT	113,125,142,170,176,197, 209,211,225	
E-ACT/ M-CTA	138,142,144,155,163,168, 218,232,241,274,283,298, 332,364,385,433	
E-ACT/ M-CTC	107,131,171,173,251, 261,403	
E-ACT/ M-CTG	117,120,147,149	
E-ACT/ M-CTT	91,94,108,125,182, 250,328	
E-AGG/ M-CAA	139,176,194,252,255, 341,412	

E-AGG/ M-CAC	88,91,95,103,112,118,146, 151,167,200	
E-AGG/ M-CAG	76,88,92,94,99,106,112,115, 133,139, 148,183	
E-AGG-/ M-CAT	82,101,111,168,248,250	
E-AGG-/ M-CTA	101,157,160,193,218,299,355	
E-AGG-/ M-CTC		75,77,100,107150, 234,288,298,396
E-AGG-/ M-CTG		58,60,81,85,95,99,111 115,173,216,233,253, 257,277
E-AAC/ M-CCT		59,69,81,86,89,160, 168,198,218,243
E-ACA/ M-CCT		119,125,147,175,288
E-ACC/ M-CCT		76,136,169,218,225, 276,289,343
E-ACG/ M-CCA		71,90,94,114,215,229, 240,256
E-ACG/ M-CCT		64,87,128,159,179
E-ACT/ M-CCT		140,235,332
E-AGC/ M-CCT		129131,154,151,163
E-AGG/ M-CCT		61,69,72,89,112,127, 131,146, 222

^a‘Mississippi Nemaheart’ and ‘Carolina Wonder’ are homozygous for the dominant *N* gene that controls resistance to *Meloidogyne incognita* in pepper (*Capsicum annuum*) and ‘Yolo Wonder B’ is homozygous recessive.

Table 2. Gall and egg mass indices, *Meloidogyne incognita* eggs per g fresh root, and fibrous root index for pepper entries with resistance to root-knot nematodes conditioned by the *N* or *Me* genes inoculated with *M. incognita* race 3 in a greenhouse test.^a

Pepper cultivar	Gall index ^b	Egg mass index ^b	Eggs/g fresh root ^c	Fibrous root index ^d
<i>N</i> gene^e				
PA-426	1.0 a ^f	1.0 a	79 ab	2.5 bc
Carolina Cayenne	1.0 a	1.0 a	214 c	2.4 a-c
Charleston Belle	1.0 a	1.0 a	91 a-c	2.3 a-c
Carolina Wonder	1.0 a	1.0 a	139 a-c	1.9 a
<i>Me</i> gene^g				
HDA 149	1.0 a	1.0 a	55 a	2.4 a-c
PM 687	1.0 a	1.0 a	96 a-c	2.1 ab
PM 217	1.8 b	1.0 a	202 bc	2.2 bc
HDA 330	2.5 c	1.3 a	1,723 d	2.3 a-c
<i>Susceptible checks</i>				
Yolo Wonder B	3.9 d	3.8 b	13,390 e	2.7 c
PA-350	4.3 e	4.3 c	61,944 f	4.1 d
California Wonder	4.4 e	4.1 bc	18,230 e	2.8 c
Keystone Resistant Giant	4.5 e	4.1 bc	16,459 e	2.6 c

^aTwo-wk-old pepper seedlings were transplanted into benches containing 2 sand : 1 soil (vol:vol) in the greenhouse on 3 March 2004. The soil surrounding each plant was infested with approximately 3,000 eggs of *M. incognita* race 3 on 12 March 2004. The experimental design was a randomized complete block with 5 replications of 5 plants/plot (n=25). Roots were lifted and scored for galling and egg mass production on 4 May 2004.

^bGall and egg mass indices: 1 = 0% to 3% root system galled or covered with egg masses, 2 = 4% to 25%, 3 = 26% to 50%, 4 = 51% to 79%, and 5 = 80% to 100% root system galled or covered with egg masses.

^cData were log₁₀(x+1) transformed before analysis. Back transformed data are shown.

^dFibrous root mass using a 1 to 5 scale where 1 = large amount of fibrous roots (best); 3 = moderate amount of fibrous roots, and 5 = no fibrous roots present (worst).

^eResistance to root-knot nematodes conferred by *N* gene.

^fMean separation within a column by Duncan's multiple range test, P<0.05.

^gResistance to root-knot nematodes conferred by *Me* gene(s).

Fig. 1

Root-gall Severity Ratings for Control Cultivars (Population I)

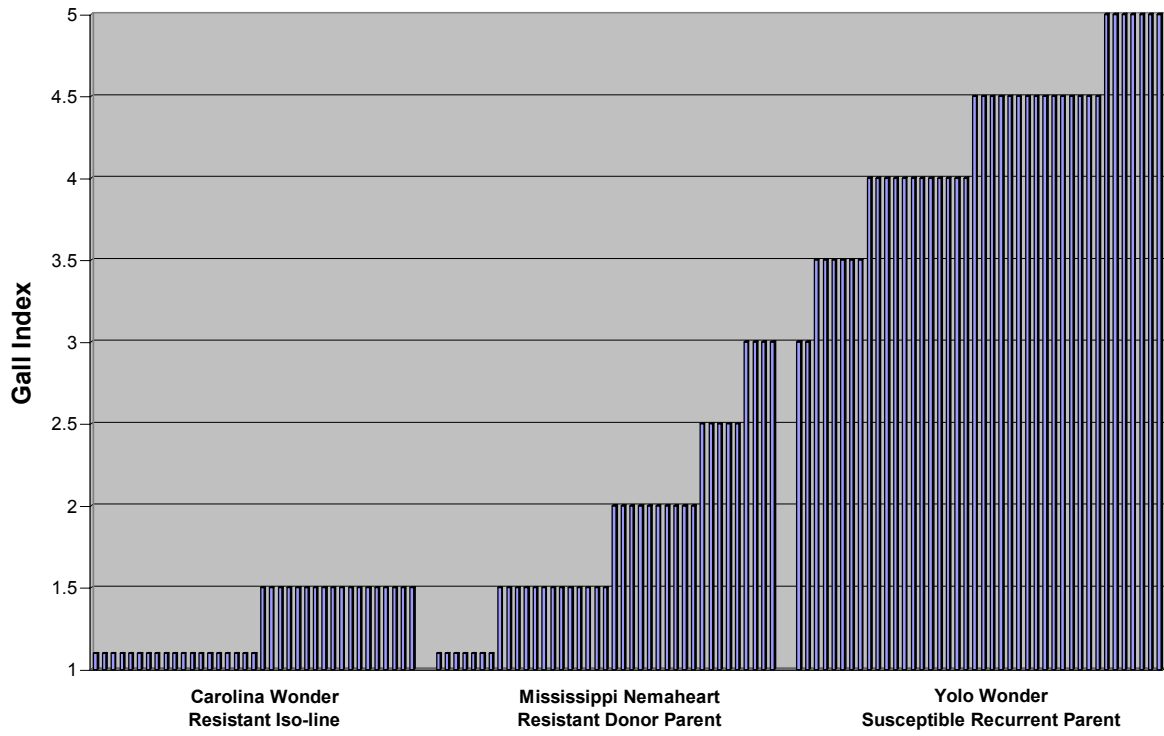


Fig. 2

Population I
Distribution of Root-knot Nematode Resistant and Susceptible Classes of F2 Plants

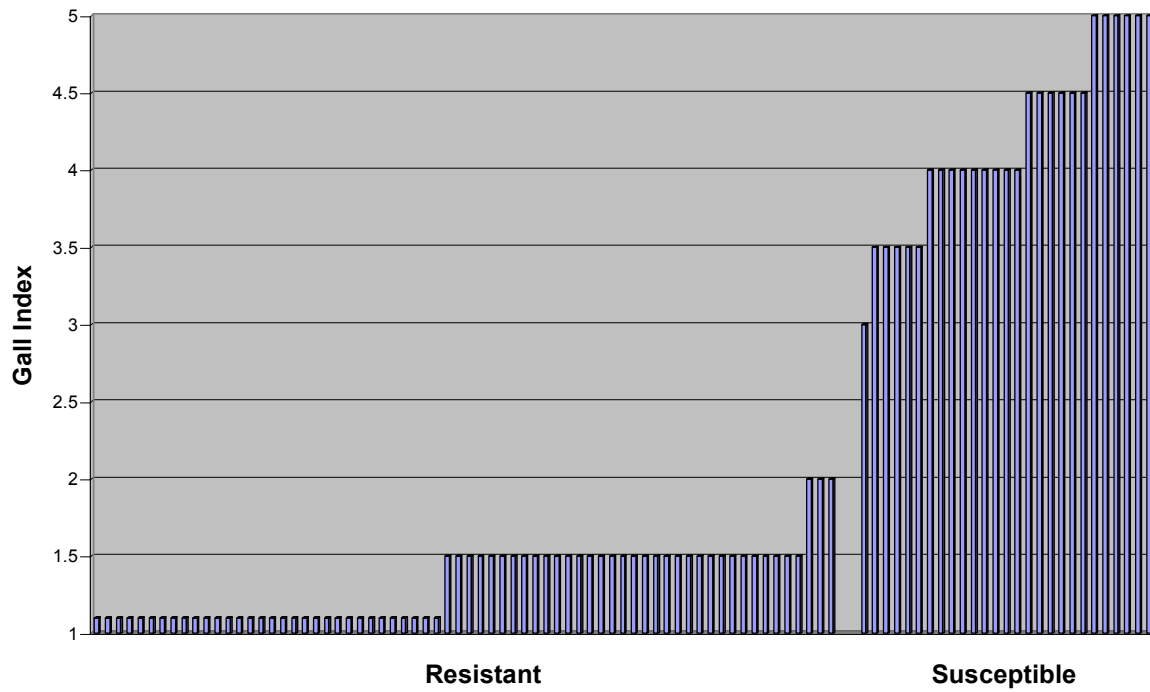


Fig. 4

Population II
Distribution of Root-knot Nematode Resistant and Susceptible Classes of F2 Plants

