Genetic Analysis of Resistance to the Southern Root-knot Nematode in *Capsicum chinense* Jacq.

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ABSTRACT. Greenhouse tests were conducted to compare the levels of resistance to the southern root-knot nematode [*Meloidogyne incognita* (Kofoed & White) Chitwood] exhibited by recently released *Capsicum chinense* Jacq. Scotch Bonnet-type germplasm lines PA-353, PA-398, and PA-426 to the levels of resistance exhibited by *C. annuum* L. 'Carolina Cayenne' and 'Mississippi Nemaheart'; to determine the inheritance of the resistance in *C. chinense* germplasm line PA-426; and to determine the genetic relationship between the resistances exhibited by *C. chinense* germplasm line PA-426 and *C. annuum* 'Carolina Cayenne'. The results of a replicated test indicated that the level of resistances exhibited by the recently released *C. chinense* germplasm lines is equal to the level of resistances exhibited by the resistant *C. annuum* cultivars. Evaluation of parental, F₁, F₂, and backcross populations of the cross PA-426 × PA-350 (a susceptible Habanero-type *C. chinense* cultigen) indicated that the resistance in *C. chinense* is conditioned by a single dominant gene. The results of an allelism test indicated that this dominant gene is allelic to the dominant gene that conditions much of the southern root-knot nematode resistance in the *C. annuum* 'Carolina Cayenne'. The ease and reliability of evaluating plants for resistance to root-knot nematodes and the availability of a simply inherited source of outstanding resistance makes breeding for southern root-knot nematode resistance a viable objective in *C. chinense* breeding programs.

Although not traditionally cultivated in the United States, pepper cultivars belonging to the species *Capsicum chinense* Jacq. are becoming popular because of the increasing popularity of pungent peppers. Two cultivar classes of *C. chinense*, Habanero and Scotch Bonnet, contain some of the world's most pungent peppers (Hazen-Hammond, 1993). *C. chinense* cultivars, like many of the *C. annuum* L. cultivars that are grown predominantly in the United States, are subject to being severely damaged by southern root-knot nematodes [*Meloidogyne incognita* (Kofoed & White) Chitwood]. Fery and Thies (1997) recently evaluated available *C. chinense* cultigens for reaction to *M. incognita*, and found that all cultigens obtained from commercial sources and all Habanero-type cultigens were susceptible. However, they did identify high levels of resistance in several Scotch Bonnet-type cultigens obtained from heirloom collections, noted that these resistant cultigens might be excellent sources of resistance for development of root-knot nematode resistant Habanero peppers, and subsequently released the resistant Scotch Bonnet-type germplasm lines PA-353, PA-398, and PA-426 (Fery and Thies, 1997, 1998).

The Scotch Bonnet-type germplasm lines PA-353, PA-398, and PA-426 hold considerable promise for use as parental materials in *C. chinense* breeding programs. The breeding value of any root-knot nematode resistant germplasm would be greatly enhanced if the resistance was adequately characterized and the mode of inheritance was understood. These needs prompted us to compare the level of resistance exhibited by the recently released *C. chinense* germplasm lines to that exhibited by resistant *C. annuum* cultivars, to determine the inheritance of resistance to *M. incognita* exhibited

by the *C. chinense* germplasm line PA-426, and to compare the genetic nature of the resistance exhibited by PA-426 to that exhibited by *C. annuum* 'Carolina Cayenne'.

Materials and Methods

The data reported here are from a series of greenhouse experiments conducted at the U.S. Vegetable Laboratory, Charleston, S.C. Seeds of all parental, F₁, F₂, and backcross generations were produced in the greenhouse using standard crossing and selfing procedures. The tests were conducted in 4.1 × 1.7 × 0.2-m benches containing a steam-pasteurized mixture of soil, sand and peatmoss (6:3:1, by volume). Seedlings were started in flats containing a sterilized artificial growth medium, and transplanted into the benches after true leaves had expanded. After the plants were established and growing, each plant was inoculated with ≈3,000 *M. incognita* race 3 eggs. The egg inoculum used for all tests was extracted from *M. incognita*-infected PA-136 cayenne pepper roots using 0.5% NaOCl (Dukes et al., 1997; Hussey and Barker, 1973).

The planting arrangement was a 10 × 12-cm rectangular pattern. To minimize the effects of moisture and temperature stress, the outer two rows around each bench were utilized as buffers. Greenhouse temperature was maintained between 24 and 32 °C and all plants were evaluated 9 to 12 weeks after inoculation. Each plant received two subjective scores, one for the prevalence of root gall and another for the prevalence of egg masses. The following scale was used to score the severity of galling: 1 = no galls; 2 = light galling, 1% to 25% of root system galled; 3 = moderate galling, 26% to 50% of root system galled; 4 = heavy galling, 51% to 75% of root system galled; and 5 = severe galling, 76% to 100% of root system galled. The number of egg masses per root system were scored as follows: 1 = no egg masses evident, 2 = scattered egg masses covering 1% to 25% of the root system, 3 = moderate number of egg masses covering 26% to 50% of root system, 4 = numerous egg masses covering 51% to 75% of root system, and 5 = extremely large numbers of egg masses covering 76% to 100% of root system. All plants with gall and egg mass indices of 1 or 2 were classified as root knot resistant; plants rated 3 to 5 for either galls or egg masses were classified as susceptible. Chi-square tests

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for goodness-of-fit were used in testing all genetic hypotheses.

CHARACTERIZATION OF RESISTANCE (EXPT. I). This experiment was conducted to compare the level of resistance to *M. incognita* exhibited by recently released *C. chinense* germplasm lines to the levels of resistance exhibited by resistant *C. annuum* cultivars and to determine the mode of gene action exhibited by F_1 hybrids between resistant and susceptible *C. chinense* lines. The experiment included the three resistant Scotch Bonnet-type *C. chinense* germplasm lines PA-353, PA-398, and PA-426 (Fery and Thies, 1997), a susceptible Habanero-type cultigen obtained from Jordan Seeds, Inc., Woodbury, Minn. (PA-343), a susceptible Habanero-type cultigen obtained from a listed member of the Seed Savers Exchange (PA-350), two commercial *C. chinense* cultivars grown in Jamaica ('West Indies Red' and 'Scotch Bonnet'), four *C. chinense* x *C. chinense* F_1 hybrids (resistant by susceptible crosses), two resistant *C. annuum* controls ('Mississippi Nemaheart' and 'Carolina Cayenne'), and two susceptible *C. annuum* controls ('California Wonder' and 'Keystone Resistant Giant'). A completely randomized experimental design was used. Ten plants per accession were planted. The seeds were planted on 26 Aug. 1996, the seedlings were transplanted on 19 Sept. 1996, established plants were inoculated on 23 Sept. 1996, and the roots of each plant were evaluated on 18 Dec. 1996. The 1.05% NaOCl procedure was used to extract *M. incognita* eggs from fibrous root samples of each plant of PA-353, PA-426, and PA-350, and the PA-353 x PA-350 and PA-426 x PA-350 hybrids (Hussey and Barker, 1973).

INHERITANCE OF RESISTANCE (EXPT. II). Plants of the parental, F_1 , F_2 , and backcross generations of the cross PA-426 x PA-350 were tested for resistance to *M. incognita*. PA-426, a Scotch Bonnet-type pepper, is resistant to *M. incognita* (Fery and Thies, 1998). PA-350, a Habanero-type pepper, is highly susceptible (Fery and Thies, 1997). The experimental design was a randomized complete block with four replications. Each plot contained five plants. Each replication contained two plots of PA-426 plants, two plots of PA-350 plants, four plots of F_1 plants (included two plots of the

reciprocal cross), eight plots of F_2 plants, eight plots of F_1 x PA-426 backcross plants, eight plots of F_1 x PA-350 backcross plants, one plot of the resistant control 'Carolina Cayenne' (Fery et al., 1986; Thies et al., 1997), and one plot of the susceptible control 'California Wonder'. The seeds were planted on 12 Mar. 1997, the seedlings were transplanted on 25 Mar. 1997, established plants were inoculated on 16 Apr. 1997, and the roots of each plant were evaluated on 24 July 1997.

ALLELISM STUDY (EXPT. III). Plants of the parental and F_2 generations of the cross PA-426 x 'Carolina Cayenne' were tested for resistance to *M. incognita*. 'Carolina Cayenne' is a highly resistant *C. annuum* cultivar whose resistance is derived from 'Carolina Hot' (Fery et al., 1986). Fery and Dukes (1996) reported that resistance in 'Carolina Hot' is governed by two genes, one dominant and one recessive; they demonstrated that the dominant gene in 'Carolina Hot' is allelic to the single dominant gene (*N*) that conditions *M. incognita* resistance in *C. annuum* 'Mississippi Nemaheart'. The experimental design was a randomized complete block. Each plot contained five plants. Each replication contained two plots of PA-426 plants, two plots of 'Carolina Cayenne' plants, and eight plots of the F_2 plants. Additionally, each replication contained two plots each of the susceptible cultivars California Wonder and Keystone Resistant Giant. 'California Wonder' and 'Keystone Resistant Giant' plants were included as controls. The seeds for this test were planted on 12 Mar. 1997, the seedlings were transplanted on 25 Mar. 1997, established plants were inoculated on 16 Apr. 1997, and the roots of each plant were evaluated on 25 July 1997.

Results and Discussion

The procedures used to infest the established plants in the growing medium with *M. incognita* eggs were effective and reliable. All homozygous lines with known reactions to the parasite reacted as expected.

Table 1. Gall indices, egg mass indices, and numbers of eggs per gram fresh root tissue for seven *Capsicum chinense* accessions, four *C. chinense* F_1 populations, and selected *C. annuum* controls inoculated with the southern root-knot nematode, *Meloidogyne incognita* (Expt. I).

Population ^a	Gall index ^b	Egg mass index ^c	No. eggs/g fresh root tissue
<i>C. chinense</i>			
PA-353 (R)	1.05 b ^w	1.05 d	159 b
PA-398 (R)	1.05 b	1.05 d	---
PA-426 (R)	1.00 b	1.00 d	193 b
PA-343 (S)	3.60 a	3.40 ab	---
PA-350 (S)	3.45 a	3.05 bc	11,465 a
West Indies Red	3.08 a	2.83 c	---
Scotch Bonnet	3.61 a	3.33 ab	---
F_1 hybrids			
PA-353 x PA-343	1.20 b	1.05 d	---
PA-353 x PA-350	1.10 b	1.00 d	273 b
PA-398 x PA-343	1.38 b	1.38 d	---
PA-426 x PA-350 ^v	1.00 b	1.00 d	341 b
<i>C. annuum</i>			
Mississippi Nemaheart (R)	1.10 b	1.10 d	---
Carolina Cayenne (R)	1.20 b	1.20 d	---
California Wonder (S)	3.65 a	3.55 a	---
Keystone Resistant Giant (S)	3.15 a	2.80 c	---

^aR = known resistant genotype; S = known susceptible genotype.

^bPlants rated on a scale of 1 to 5; 1 = no galling and 5 = galls covering at least 80% of root system.

^cPlants rated on a scale of 1 to 5; 1 = no egg masses and 5 = egg masses covering at least 80% of root system.

^wMean separation within columns at $P \leq 0.01$ by Student-Newman-Keuls multiple range test.

^vThis population contained a single, susceptible plant. Plant was assumed to be the result of a seed mixture or planting error and was discarded.

Table 2. Segregation for reaction to the southern root-knot nematode (*Meloidogyne incognita*) in parental, F₁, F₂, and backcross generations of the cross PA-426 x PA-350 (Expt. II).

Population	No. of plants		Expected ratios (R:S) ^y	χ^2	P
	Resistant	Susceptible			
PA-426	40		All R	---	---
PA-350		38	All S	---	---
F ₁	18 ^y		All R	---	---
F ₂	119	40	3:1	0.002	0.99–0.95
F ₁ x PA-426	160		All R	---	---
F ₁ x PA-350	72	56	1:1	2.000	0.20–0.05
Carolina Cayenne ^x	20		All R	---	---
California Wonder ^w		20	All S	---	---

^yR = resistant (gall index ≤ 2), S = susceptible (gall index ≥ 3).

^yNo maternal effect observed (PA-426 was maternal parent of 10 plants; PA-350 was maternal parent of 8 plants).

^xResistant *Capsicum annuum* control.

^wSusceptible *C. annuum* control.

CHARACTERIZATION OF RESISTANCE (EXPT. I). The level of resistance exhibited by the recently released *C. chinense* germplasm lines PA-353, PA-398, and PA-426 is equal to the level of resistance exhibited by the resistant *C. annuum* cultivars Mississippi Nemaheart and Carolina Cayenne (Fery and Dukes, 1996; Hare, 1957, 1966). The gall indices and egg mass indices of the resistant *C. chinense* germplasm lines were not different from those of the resistant *C. annuum* cultivars (Table 1). Examination of the gall indices, egg mass indices, and numbers of eggs per g of fresh root tissue for the F₁ hybrids indicates that resistance to the southern root-knot nematode is inherited in a completely dominant manner in *C. chinense*. In all cases, the resistance exhibited by the hybrids was equal to the resistance exhibited by the resistant parent.

INHERITANCE OF RESISTANCE (EXPT. II). PA-426 and PA-350 plants reacted to *M. incognita* as expected (Table 2). PA-426 exhibited a high level of resistance. The roots of most of the PA-350 plants exhibited heavy to severe galling and egg masses were numerous. Since preliminary analysis revealed no significant differences between the reciprocal F₁ crosses, these populations were pooled for genetic analyses. The frequency of phenotypes in the progeny generations indicated that the *M. incognita* resistance in PA-426 is conditioned by a single dominant gene. All 18 F₁ plants were resistant and all of the 160 F₁ x PA-426 backcross plants were of the expected resistant phenotype. The F₂ segregated 3 resistant : 1 susceptible, and the F₁ x PA-350 backcross segregated 1 resistant : 1 susceptible.

ALLELISM STUDY (EXPT. III). All of the plants of the parental lines of the PA-426 x 'Carolina Cayenne' cross were resistant and all of

the 'California Wonder' and 'Keystone Resistant Giant' control plants were susceptible (Table 3). The lack of segregation in the F₂ generation of this cross indicates that the dominant resistance gene in the *C. chinense* germplasm line PA-426 is allelic to the dominant resistance gene in 'Carolina Cayenne'. Thus, the dominant resistance gene in PA-426 is probably the same gene (*N*) that Fery and Dukes (1996) concluded was responsible for the bulk of the *M. incognita* resistance in the *C. annuum* germplasm they studied.

Several published reports indicate that there are a number of other genes in *Capsicum* spp. conditioning resistance to various species of root-knot nematodes (*Meloidogyne* spp.). Peter et al. (1984) suggested that resistance to *M. incognita* in Indian hot peppers is recessive. Hendy et al. (1985b) identified five genes in *C. annuum*, each conditioning resistance to one or more *Meloidogyne* species. Hendy et al. (1985a) observed the presence of additive effects of multiple genes conditioning some types of resistances. Di Vito et al. (1985) concluded that resistance in *C. frutescens* L. is monogenic dominant, but the resistance exhibited by *C. annuum* is monogenic recessive. Recent work by Di Vito et al. (1993) demonstrated the complex nature of resistance to root-knot nematodes in *Capsicum*: a single dominant gene conditioned resistance in *C. frutescens* to *M. incognita*, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood; a single dominant gene in *C. chacoense* Hunz. conditioned resistance to *M. incognita* and *M. arenaria*, but two dominant genes conditioned resistance to *M. javanica*; and a single dominant gene in *C. chinense* conditioned resistance to *M. incognita* and *M. javanica*, but two duplicate genes conditioned resistance to *M. arenaria*. Although the

Table 3. Segregation for reaction to the southern root-knot nematode (*Meloidogyne incognita*) in parental and F₂ generations of the interspecific cross *Capsicum chinense* germplasm line PA-426 x *C. annuum* 'Carolina Cayenne' (Expt. III).

Population	Gall index ^z								
	1	1+ ^y	2	2+	3	3+	4	4+	5
	No. of plants								
PA-426	39	1							
Carolina Cayenne	37	1	2						
F ₂	155	2	2						
California Wonder ^x					2	11	21	6	
Keystone Resistant Giant ^x					2	5	21	11	1

^zGall index: resistant $\leq 2+$, susceptible ≥ 3 .

^y(+)A score intermediate between two reaction classes.

^xSusceptible *C. annuum* control.

results of the studies reported here demonstrate clearly that the single dominant gene in the *C. chinense* germplasm line PA-426 conditioning resistance to *M. incognita* is allelic to the dominant *N* gene in *C. annuum* discussed by Hare (1957) and Fery and Dukes (1996), additional studies are needed to clarify the relationship between this gene and the other root-knot nematode resistance genes described in the *Capsicum* genetics literature.

Conclusions

The results of this study confirm that the level of resistance exhibited by the recently released *C. chinense* germplasm lines PA-353, PA-398, and PA-426 to the southern root-knot nematode is equal to the high level of resistance exhibited by *C. annuum* 'Carolina Cayenne' and 'Mississippi Nemaheart'. A single dominant gene conditions the high level of resistance to the southern root-knot nematode exhibited by the *C. chinense* germplasm line PA-426, and the results of an allelism test indicate that this gene is likely that same dominant gene (*N*) that is responsible for much of the resistance exhibited by 'Carolina Cayenne.' The ease and reliability of evaluating plants for resistance to root-knot nematodes and the availability of a simply inherited source of outstanding resistance makes breeding for southern root-knot resistance a viable objective in *C. chinense* pepper breeding programs. This objective should be attained easily by the application of conventional plant breeding methodologies.

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