Inheritance of Resistance to the Peanut Root-knot Nematode in Capsicum chinense

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Abstract. Greenhouse experiments determined the inheritance of resistance to the peanut root-knot nematode [Meloidogyne arenaria (Neal) Chitwood] in Capsicum chinense Jacq. germplasm lines PA-353 and PA-426. Evaluation of parental, F\(_1\), F\(_2\), and backcross populations of the crosses PA-353 x PA-350 and PA-426 x PA-350 (PA-350 is a susceptible cultigen) indicated that resistance in both C. chinense germplasm lines was conditioned by a single dominant gene. Evaluation of the F\(_1\) x resistant parent backcross populations in the cytoplasm of their respective resistant and susceptible parents indicated that the cytoplasm of the resistant parent is not needed for full expression of resistance. Allelism tests indicated that the dominant resistance gene in both PA-353 and PA-426 is allelic to a resistance gene in C. annuum L. ‘Carolina Cayenne’. However, these allelism tests did not demonstrate conclusively that the M. arenaria race 1 resistance gene in C. chinense is the N gene that conditions resistance to the southern root-knot nematode [Meloidogyne incognita (Kofoid & White) Chitwood] in C. annuum. The ease and reliability of evaluating plants for resistance to root-knot nematodes and the availability of simply inherited sources of resistance makes breeding for peanut root-knot nematode resistance a viable objective in C. chinense breeding programs.

Although cultivars belonging to the species Capsicum annuum account for most of the peppers grown in the United States, cultivars belonging to the species C. chinense are becoming increasingly popular. Two cultivar classes of C. chinense, Scotch Bonnet and Habanero, are now common in the United States. Capsicum chinense cultivars, like C. annuum cultivars, can be severely damaged by several species of the root-knot nematode (Meloidogyne Goeldi sp.). Recent research by Fery and Thies (1997) demonstrated that all C. chinense cultivars available to U.S. growers are susceptible to the southern root-knot nematode (Meloidogyne incognita). However, they identified several cultivars from heirloom collections that are resistant to M. incognita, and subsequently released the highly resistant Scotch Bonnet-type germplasm lines PA-353, PA-398, and PA-426 (Fery and Thies, 1997, 1998a). Fery and Thies (1998b) also studied inheritance of southern root-knot nematode resistance in Scotch Bonnet-type C. chinense germplasm and determined that the resistance is conditioned by a single dominant gene.

Preliminary research by the authors (unpublished data) with the two known races of the peanut root-knot nematode (M. arenaria) indicates that only race 1 is highly pathogenic on C. chinense. Our research also indicated that the southern root-knot nematode resistant germplasm lines PA-353, PA-398, and PA-426 are also highly resistant to M. arenaria race 1. Meloidogyne arenaria is one of the four Meloidogyne species that has numerous crop plants as hosts, and it has a geographic distribution in the southern United States that is similar to that of M. incognita (Sasser and Carter, 1982). Research results in several published reports suggest that the single dominant gene in C. chinense identified by Fery and Thies (1998b) to condition resistance to M. incognita might not be responsible for conditioning the resistance to M. arenaria race 1. Di Vito et al. (1985) showed that the C. chinense ‘Surrinam 8’ is resistant to M. incognita and M. javanica (Treub) Chitwood (the javanese root-knot nematode), but not to M. arenaria. Later, Di Vito et al. (1993) and Di Vito and Saccardo (1996) demonstrated that resistances in C. chinense line 56-547/7 to M. incognita and M. javanica are conditioned by single dominant genes, but that resistance in the line to M. arenaria is conditioned by two duplicate dominant genes.

The root-knot nematode resistant C. chinense germplasm lines released by Fery and Thies (1998a) hold promise for pepper breeding programs to develop C. chinense cultivars with broad resistance to root-knot nematodes. However, the breeding value of these nematode resistant lines would be greatly enhanced if the modes of inheritance to all pertinent Meloidogyne species were understood. These needs prompted us to determine the inheritance of resistance to M. arenaria race 1 in the C. chinense germplasm, and to compare the genetic nature of the resistance exhibited by this germplasm to that exhibited by the C. annuum ‘Carolina Cayenne’.

Materials and Methods

The data reported are from a series of greenhouse experiments conducted at the U. S. Vegetable Laboratory, Charleston, S.C. Seeds of all parental, F\(_1\), F\(_2\), and backcross populations were produced in a greenhouse using standard crossing and selfing procedures. The tests were conducted in 4.1 × 1.7 × 0.2-m benches containing a steam-pasteurized medium of 6 soil : 3 sand : 1 peat (by volume). A slow-release, complete analysis fertilizer was incorporated into the medium, and the reaction was adjusted to pH 6.0. Seeds were germinated in flats containing a sterilized artificial growth medium, and seedlings were transplanted into the benches after true leaves had expanded. After the plants were established and growing, 5 mL tap water containing 3,000 M. arenaria race 1 eggs were pipetted around the base of each plant.
Meloidogyne arenaria race 1 (obtained from S. L. Lewis, Clemson Univ., Clemson, S.C.) was maintained as an isolated culture on ‘Rutgers’ tomato (Lycopersicon esculentum Mill.) in the greenhouse. The inoculum used for all tests was extracted from M. arenaria-infected tomato roots using 0.5% NaOCl (Hussey and Barker, 1973).

The planting arrangement was a 10 × 12-cm rectangular pattern (spacing between plants within rows: 10 cm; spacing between rows: 12 cm). To minimize the effect of moisture and temperature stress, the outer two rows of plants on the perimeter of each bench were utilized as buffers. Greenhouse air temperatures were maintained between 24 and 32 °C. All plants were evaluated 13 to 14 weeks after inoculation. Each plant received two subjective scores, one for the prevalence of root galling and another for the prevalence of egg masses. The following scale was used to score 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 80% of root system galled; and 5 = severe galling, 81% 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 the root system; 4 = numerous egg masses covering 51% to 80% of the root system; and 5 = extremely large numbers of egg masses covering 81% to 100% of the root system. All plants with gall severity scores of 1 or 2 were classified as resistant; plants scored 3 to 5 were classified as susceptible. Gall index, egg-mass index, and egg count data were analyzed using analysis of variance procedures, and means were separated using Duncan’s multiple range test at \( P \leq 0.01 \).

**Chi-square tests for goodness-of-fit were used in testing genetic hypotheses.**

**INHERITANCE OF RESISTANCE (EXPTS. 1A AND B).** Plants of the parental, F1, F2, and backcross populations of the crosses PA-353 × PA-350 (Expt. 1A) and PA-426 × PA-350 (Expt. 1B) inoculated with the peanut root-knot nematode (Meloidogyne arenaria race 1).

| Population | Gall index | Egg-mass index | No. eggs/ g fresh root tissue |
|------------|------------|----------------|-----------------------------|
| Expt. 1A   |            |                |                             |
| PA-353 (P1) | 1.00 b ⁴  | 1.00 b ⁴       | 242 b                       |
| PA-350 (P2) | 4.50 a     | 4.12 a         | 107,506 a                   |
| F1 (P1 × P2) | 1.00 b ⁴  | 1.00 b ⁴       | 391 b                       |
| Expt. 1B   |            |                |                             |
| PA-426 (P3) | 1.00 b ⁴  | 1.00 b ⁴       | 415 b                       |
| PA-350 (P2) | 4.66 a     | 4.70 a         | 56,837 a                    |
| F1 (P3 × P2) | 1.12 b ⁴  | 1.12 b ⁴       | 896 b                       |

⁴Plants rated on a scale of 1 to 5; 1 = no galling and 5 = galls covering at least 81% of root system.

⁵Plants rated on a scale of 1 to 5; 1 = no egg masses and 5 = egg masses covering at least 81% of root system.

Mean separation within columns for an experiment by Duncan’s multiple range test, \( P \leq 0.01 \).

**Table 1. Gall indices, egg-mass indices, and number of eggs per gram fresh root tissue for parental and F1 populations of the crosses PA-353 x PA-350 (Expt. 1A) and PA-426 x PA-350 (Expt. 1B) inoculated with the peanut root-knot nematode (Meloidogyne arenaria race 1).**

Meloidogyne arenaria race 1 (obtained from S. L. Lewis, Clemson Univ., Clemson, S.C.) was maintained as an isolated culture on ‘Rutgers’ tomato (Lycopersicon esculentum Mill.) in the greenhouse. The inoculum used for all tests was extracted from M. arenaria-infected tomato roots using 0.5% NaOCl (Hussey and Barker, 1973).

The planting arrangement was a 10 × 12-cm rectangular pattern (spacing between plants within rows: 10 cm; spacing between rows: 12 cm). To minimize the effect of moisture and temperature stress, the outer two rows of plants on the perimeter of each bench were utilized as buffers. Greenhouse air temperatures were maintained between 24 and 32 °C. All plants were evaluated 13 to 14 weeks after inoculation. Each plant received two subjective scores, one for the prevalence of root galling and another for the prevalence of egg masses. The following scale was used to score 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 80% of root system galled; and 5 = severe galling, 81% 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 the root system; 4 = numerous egg masses covering 51% to 80% of the root system; and 5 = extremely large numbers of egg masses covering 81% to 100% of the root system. All plants with gall severity scores of 1 or 2 were classified as resistant; plants scored 3 to 5 were classified as susceptible. Gall index, egg-mass index, and egg count data were analyzed using analysis of variance procedures, and means were separated using Duncan’s multiple range test at \( P \leq 0.01 \). Chi-square tests for goodness-of-fit were used in testing genetic hypotheses.

**Fig. 1. Examples of effects of the peanut root-knot nematode (Meloidogyne arenaria race 1) on the parental and F1 hybrid plants from (A) Expt. 1A and (B) Expt. 1B. (A) Root systems of the resistant germplasm line PA-353 (left), the resistant F1 of PA-353 x PA-350 (center), and the susceptible germplasm line PA-350 (right). (B) Root systems of the resistant germplasm line PA-426 (left), the resistant F1 of PA-426 x PA-350 (center), and the susceptible germplasm line PA-350 (right). Note the lack of galls on the healthy root systems of the resistant parents and F1 hybrids and the presence of numerous galls and extensive stunting of the susceptible PA-350 root systems.**

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Table 2. Segregation for resistance to the peanut root-knot nematode (Meloidogyne arenaria race 1) in parental, F₁, F₂, and backcross populations of the crosses PA-353 × PA-350 (Expt. 1A) and PA-426 × PA-350 (Expt. 1B).

| Population | No. plants in classes | Expected ratios (R:S) | Chi square | P |
|------------|------------------------|-----------------------|-----------|---|
| Expt. 1A   |                        |                       |           |   |
| PA-353 (P₁) | 28 | All R | --- | --- |
| PA-350 (P₂) | 27 | All S | --- | --- |
| F₁ (P₁ × P₂) | 20 | All R | --- | --- |
| F₂ (P₁ × P₂) | 96 | 44 | 3:1 | 3.08 | 0.08 |
| F₂ x PA-353 | 149 | All R | --- | --- |
| F₂ x PA-350 | 77 | 57 | 1:1 | 2.98 | 0.08 |
| Expt. 1B   |                        |                       |           |   |
| PA-426 (P₁) | 40 | All R | --- | --- |
| PA-350 (P₂) | 40 | All S | --- | --- |
| F₁ (P₁ × P₂) | 11 | All R | --- | --- |
| F₂ (P₁ × P₂) | 117 | 41 | 3:1 | 0.08 | 0.78 |
| F₂ x PA-426 | 159 | All R | --- | --- |
| F₂ x PA-350 | 78 | 78 | 1:1 | 0.00 | 1.00 |

*R = resistant (gall index ≤ 2), S = susceptible (gall index ≥ 3).

Inheritance of resistance (Expts. 1A and B). Examination of the gall indices, egg-mass indices, and the numbers of eggs per gram of fresh root tissue for the F₁ populations indicated that resistance to *M. arenaria* race 1 in PA-353 and PA-426 is inherited in a completely dominant manner (Table 1). In all cases, the resistance exhibited by the F₁ population was equal to that exhibited by the resistant parent (Fig. 1). The frequency of phenotypes in the progeny populations of Expt. 1A and Expt. 1B indicated that the *M. arenaria* race 1 resistance is conditioned by a single dominant gene in both PA-353 and PA-426 (Table 2). All F₁ plants were resistant and all of the F₁ × resistant parent backcross plants were of the expected resistant phenotype. The F₂ populations segregated 3 resistant : 1 susceptible, and the F₁ × susceptible parent backcross populations segregated 1 resistant : 1 susceptible.

Although seed shortages prohibited evaluation of reciprocal F₁ populations in Expts. 1A and B, supplemental studies were conducted concurrent with Expt. 1B to evaluate both of the F₁ × resistant parent backcross populations in the cytoplasms of their respective resistant and susceptible parents (data not presented). All of these backcross populations segregated highly resistant plants, indicating that neither the PA-353 or PA-426 cytoplasms are needed for full expression of resistance.

Allelism studies (Expts. 2A and B). All of the plants in the parental and F₂ populations of the crosses PA-353 × ‘Carolina Cayenne’ (Expt. 2A) and PA-426 × ‘Carolina Cayenne’ (Expt. 2B) were tested for resistance to *M. arenaria* race 1. ‘Carolina Cayenne’ is a *C. annuum* cultivar that is highly resistant to several root-knot nematode species, including *M. arenaria*, *M. incognita*, and *M. javanica* (Fery et al., 1986; Noe, 1992; Thies et al., 1997; Thies and Fery, 2000). The resistance in ‘Carolina Cayenne’ to *M. incognita* was derived by pure-line selection from ‘Carolina Hot’ (Fery et al., 1986); resistance in ‘Carolina Hot’ to *M. incognita* is conditioned by two genes, one dominant and one recessive (Fery and Dukes, 1996). The experimental design for both Expt. 2A and B was a randomized complete block with four replications. Each plot contained five plants. Each replicate contained two plots of each parental population and eight plots of the F₂ population. Additionally, each replicate in Expt. 2A contained two control plots of the susceptible, bell-type *C. annuum* ‘California Wonder’ and each replicate in Expt. 2B contained two control plots of the susceptible, Habanero-type *C. chinense* cultivar PA-350. In 1998, the seeds for Expt. 2A were sown 14 Jan., the seedlings were transplanted 3 Feb., established plants were inoculated 3 Mar., and the roots of each plant were evaluated 10 June. In 1999, the seeds for Expt. 2B were sown 15 Jan., the seedlings were transplanted 2 Feb., established plants were inoculated 23 Feb., and the roots of each plant were evaluated 25 May.

Results and Discussion

All of the parental lines reacted as expected. All of the plants of the susceptible parents exhibited strong susceptible reactions, and all of the plants of the resistant parents exhibited highly resistant reactions.

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Table 3. Segregation for reaction to the peanut root-knot nematode (*Meloidogyne arenaria* race 1) in parental and *F₂* populations of the interspecific crosses *Capsicum chinense* germplasm line PA-353 × *C. annuum* ‘Carolina Cayenne’ (Expt. 2A) and *C. chinense* germplasm line PA-426 × *C. annuum* cv. Carolina Cayenne (Expt. 2B).

| Population                      | No. plants in each gall-index class
|---------------------------------|---------------------------------|
|                                 | 1     | 2     | 3     | 4     | 5     |
| **Expt. 2A**                    |       |       |       |       |       |
| PA-353 (P₁)                    | 35    |       |       |       |       |
| Carolina Cayenne (P₁)          | 33    |       |       |       |       |
| *F₂* (P₁ x P₁)                 | 156   | 1     |       |       |       |
| California Wonder*             | 24    | 10    | 4     |       |       |
| **Expt. 2B**                    |       |       |       |       |       |
| PA-426 (P₁)                    | 59    |       |       |       |       |
| Carolina Cayenne* (P₁)         | 58    |       |       |       |       |
| *F₂* (P₁ x P₁)                 | 199   |       |       |       |       |
| PA-426*                         |       | 4     | 30    | 26    |       |

*Gall index: resistant ≤ 2, susceptible ≥ 3.*

*Susceptible *C. annuum* control.

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

*Susceptible *C. chinense* control.

the single dominant gene in the *C. chinense* germplasm lines conditioning the *M. arenaria* race 1 resistance is the same gene (*N*) that Fery and Dukes (1996) and Fery and Thies (1998b) concluded was likely responsible for the bulk of the *M. incognita* resistance in the *C. annuum* and *C. chinense* germplasm they studied. However, another gene common to both *C. annuum* and *C. chinense* might be responsible for the *M. arenaria* race 1 resistance exhibited by the *C. chinense* germplasm lines evaluated in this study. Not only did we observe *M. arenaria* race 1 resistant plants in the ‘California Wonder’ population (presumed *nh* genotype) used as a control in Expt. 2A, but the work by Di Vito and his colleagues discussed above (Di Vito et al., 1985, 1993; Di Vito and Saccardo, 1996) provides examples of a *M. incognita* resistant *C. chinense* cultivar that is susceptible to *M. arenaria* and a *C. chinense* germplasm line with *M. arenaria* resistance conditioned by more than one dominant gene.

Our findings demonstrate that a single dominant gene conditions the high level of resistance to *M. arenaria* race 1 exhibited by the *C. chinense* germplasm lines PA-353 and PA-426. Results of allelism tests indicate that the resistance gene in each of these germplasm lines is allelic to a gene in *C. annuum* ‘Carolina Cayenne’. However, further research is needed to determine whether this dominant *M. arenaria* race 1 resistance gene in *C. chinense* is the *N* gene that conditions the *M. incognita* resistance in *C. annuum*. The availability of simply-inherited sources of resistance makes breeding for *M. arenaria* race 1 resistance a viable objective in *C. chinense* breeding programs. This objective should be readily obtained by the application of conventional plant breeding methodologies.

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