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Resistance to *Meloidogyne* spp. in Allohexaploid Wheat Derived from *Triticum turgidum* and *Aegilops squarrosa*[1]

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Abstract: Expression of resistance to *Meloidogyne incognita* and *M. javanica* from *Aegilops squarrosa* was studied in a synthetic allohexaploid produced from *Triticum turgidum* var. *durum* cv. Produra and *Ae. squarrosa* G 3489. The reproductive rate of different races of *M. incognita* and *M. javanica*, expressed in eggs per gram of fresh root, was low ($P < 0.05$) on the synthetic allohexaploid and the resistant parent, *Ae. squarrosa* G 3489, compared with different bread and durum wheat cultivars. Reproduction of race 2 and race 3 of *M. incognita* and an isolate of *M. javanica* was studied on the synthetic allohexaploid and seven cultivars of *T. aestivum*: Anza, Coker 747, Coker 68-15, Delta Queen, Double Crop, McNair 1813, and Southern Bell. The latter six cultivars are grown in the southeastern United States and reportedly were resistant to *M. incognita*. Significant differences ($P < 0.05$) were detected in nematode reproduction on the seven bread wheat cultivars. Reproduction of *M. incognita* race 3 and *M. javanica* was highest on Anza. Reproductive rates on the six southeastern United States bread wheat cultivars varied both within and among nematode isolates. The lowest reproductive rates of the three root-knot isolates were detected in the synthetic allohexaploid.

Key words: *Aegilops squarrosa*, *Meloidogyne incognita*, *M. javanica*, resistance, root-knot nematode, *Triticum aestivum*, *T. turgidum* var. *durum*, wheat.

Meloidogyne incognita (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood can invade and develop on bread wheat (*Triticum aestivum* L. em. Thell.) and durum wheat (*T. turgidum* L. var. *durum* Desf.) (14,15). Furthermore, both *M. incognita* and *M. javanica* had high reproductive rates on California commercial wheat cultivars under controlled greenhouse conditions, but wheat was tolerant to attack by these two species (14). Recently, parasitism of wheat by *M. javanica* was reported from Brazil (18), India (13), and Egypt (A. A. Al-Sayed, pers. comm.). Relatively high soil temperatures during the wheat growing season in these tropical and subtropical countries result in high reproductive rates and damaging population levels of *M. javanica* and suppression of wheat growth and yield.

A search for sources of resistance to *Meloidogyne* spp. in both domestic and wild wheat germplasm identified an accession of *Aegilops squarrosa* L. (= *T. tauschii* (Coss) Schmal.) G 3489 from Afghanistan, resistant to several isolates of both *M. incognita* and *M. javanica* (15). The diploid (2n = 2x = 14) *Ae. squarrosa* is the probable D genome donor to the hexaploid (2n = 6x = 42) bread wheat *T. aestivum*, with the genomic constitution AABBDD (8,10,11). Desirable agronomic traits can be transferred from *Ae. squarrosa* to wheat by either the formation of an allohexaploid of *Ae. squarrosa* and tetraploid durum wheat (2n = 4x = 28, genomic constitution AABB) or by the substitution of an *Ae. squarrosa* chromosome segment for a related wheat chromosome segment (12,17). A synthetic allohexaploid (G 4299) was developed from a cross between *T. turgidum* var. *durum* cv. Produra, a commercial durum wheat cultivated in California, and root-knot nematode resistant *Ae. squarrosa* G 3489 (19).

Although commercial wheats grown in California were susceptible to both *M. incognita* and *M. javanica*, commercial wheats commonly grown in the southeastern United States were reportedly resistant to a single isolate of *M. incognita* (1).

The objectives of these studies were to determine whether 1) the resistance to *M. incognita* and *M. javanica* derived from *Ae. squarrosa* is expressed in the synthetic allohexaploid; 2) the two species, *M. incognita* and *M. javanica*, differed in their ability to

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reproduce on durum and bread wheats; 3) races of *M. incognita* differed in their ability to reproduce on wheat cultivars; and 4) there is variability in reaction to *M. incognita* and *M. javanica* in hexaploid wheat cultivars from the southeastern United States.

Materials and Methods

Nematode isolates: Cultures of *M. incognita* race 1 and race 3 and *M. javanica* were started from single egg masses on tomato (*Lycopersicon esculentum* Mill. cv. Tropic) grown in a greenhouse in 15-cm-d pots containing steam sterilized loamy sand (93% sand, 4% silt, 3% clay). *Meloidogyne incognita* race 1 originally was isolated from fig (*Ficus carica* L.) roots at the University of California, Riverside Experimental Station. Race 3 was isolated from cotton (*Gossypium hirsutum* L.) roots in Tulare County, California. *M. javanica* was originally isolated from cowpea (*Vigna unguiculata* (L.) Walp.) plants growing in Chino, California. Isolates of three *M. incognita* races—race 1 population NCSU #54, race 2 population NCSU #E1135, and race 3 population NCSU #108—originally obtained from North Carolina State University, Raleigh, were also maintained on tomato in the greenhouse. All isolates were identified morphologically and by the North Carolina differential host test (16). Egg inocula were prepared by macerating tomato plant roots in bleach solution (7).

Plant material: Seed of *Aegilops* accessions and wheat cultivars used in this study were obtained from two sources. *Aegilops squarrosa* G 1279 and G 3489, *Triticum aestivum* cultivars Anza and Chinese Spring and synthetic allohexaploid G 4299, and *T. turgidum* var. *durum* cultivars Cocorit and Produra were obtained from the University of California, Riverside, wheat germplasm collection. The *T. aestivum* cultivars Coker 747 CI 173489, Coker 68-15 CI 15291, Delta Queen CI 17893, Double Crop CI 17349, McNair 1813 CI 15289, and Southern Bell CI 17894 were obtained from the National Small Grain Collection, USDA, Beltsville, Maryland.

Seeds were surface sterilized by rinsing in 95% alcohol for 1 minute and soaking in 1% NaOCl solution for 3 minutes, rinsed three times in sterile water, and germinated at 25 C on moist filter paper in petri dishes. When the coleoptyle emerged, seedlings were planted singly 2-cm deep in 10-cm-d fiber pots containing 500 cm^3 steam sterilized loamy sand.

In all experiments, 2-week-old seedlings were inoculated with a suspension of 5,000 eggs pipetted into the root zone via three holes around the plant in each pot. Tropic tomato plants were inoculated as susceptible controls to test inoculum viability. Plants were supplemented weekly with 30 ml of 20-20-20 Plus Nutriculture (Plant Marvel, Chicago, IL) solution and maintained at 23–26 C soil temperature. Plants were harvested after accumulating approximately 1,100 degree days (base threshold 10 C). Root systems were washed free of soil, damp dried with paper towels, and weighed. Eggs were collected from root systems by the bleach technique (7), and subsamples were counted.

Experiment 1: To test the expression of resistance from *Ae. squarrosa,* seedlings of synthetic allohexaploid G 4299 were planted along with Produra and *Ae. squarrosa* G 3489, susceptible and resistant parents, respectively. In addition, Chinese Spring, for use in cytogenetic studies, was evaluated for its reaction to *Meloidogyne* spp. The seedlings were inoculated with *M. incognita* race 1 or race 2 or with *M. javanica.* Plants were arranged on greenhouse benches in a randomized block design with five replicates.

Experiments 2 and 3: Reproduction on selected wild and domesticated wheats of different isolates of three races of *M. incognita* and an isolate of *M. javanica* was tested in a greenhouse in two experiments. Synthetic allohexapaloid G 4299 and its parents were also included. In experiment 2, wheat seedlings were inoculated with *M. javanica* or *M. incognita* race 1, race 2, or race 3. In experiment 3, wheat seedlings were inoculated with *M. incognita* race 1 or race 3. Plants were arranged on green-

TABLE 1. Reproduction (eggs/g fresh root) of *Meloidogyne incognita* and *M. javanica* on wheat.

	M. incognita		
	Race 1	Race 2†	*M. javanica*
Aegilops squarrosa (2n = 14)			
G 3489	349 ab	388 b	195 b
T. turgidum L. var. *durum* (2n = 28)			
cv. Produra	1,007 ab	1,574 a	7,970 a
Triticum aestivum L. (2n = 42)			
cv. Chinese Spring	1,685 a	1,090 ab	4,207 a
Synthetic allohexaploid G 4299 (Produra × G 3489)	97 b	507 ab	174 b

Each value is the mean of five replicates.
Means followed by the same letter within a column are not different based on Duncan's multiple-range test ($P < 0.05$) performed on $\log_{10}(x + 1)$ transformed data.
† This isolate from North Carolina State University Collection.

house benches in a randomized block design with five replicates.

Experiment 4: Eight hexaploid *T. aestivum* genotypes—Anza (a high yielding locally grown cultivar), Coker 747, Coker 68-15, Delta Queen, Double Crop, McNair 1813, and Southern Bell (all six grown in the southeastern United States) and the synthetic allohexaploid G 4299—were selected for this study. Seedlings were inoculated with *M. incognita* race 2 or race 3 or *M. javanica.* The plants were arranged in a randomized block experimental design and replicated five times.

RESULTS

Experiment 1: All three isolates of *M. incognita* and *M. javanica* developed and reproduced to some extent on all the tested wheat genotypes (Table 1). Reproduction of *M. incognita* race 1 was low on synthetic allohexaploid G 4299 and on *Ae. squarrosa* G 3489 but not significantly different from that on the durum and bread wheat cultivars (Table 1). Reproduction of *M. incognita* race 2 exceeded that of race 1 on the synthetic allohexaploid and on both parents. *M. javanica* produced significantly ($P < 0.05$) more eggs on both Produra and Chinese Spring than on G 3489 or the synthetic allohexaploid G 4299.

Experiments 2 and 3: In experiment 2, *M. javanica* reproduction was overall higher than that of the three races of *M. incognita* (Table 2). Although *M. javanica* reproduced extensively on most of the wheat cultivars tested, the egg production rate was significantly ($P < 0.05$) lower on both synthetic allohexaploid G 4299 and *Ae. squarrosa* G 3489, confirming the results in experiment 1. The reproductive rates of the three races of *M. incognita* were significantly lower on the synthetic allohexaploid and *Ae. squarrosa* G 3489 than on susceptible *Ae. squarrosa* G 1279 and commercial durum and bread wheat cultivars. In experiment 3, both *M. incognita* race 1 and race 3 reproduced more on the tested wheat cultivars than in experiment 2. Reproduction was significantly lower on *Ae. squarrosa* G 3489 and the synthetic allohexaploid G 4299 than on the susceptible cultivars, again confirming the results in experiments 1 and 2 (Table 2). Chinese Spring supported intermediate levels of egg production by the two *M. incognita* races. Within each experiment, the reproductive rates of different races of *M. incognita* were similar on a given wheat genotype.

Experiment 4: Significant differences ($P < 0.05$) were detected in nematode reproduction on seven cultivars of *T. aestivum* in experiment 4 (Table 3). Reproduction of both *M. incognita* race 3 and *M. javanica* was significantly ($P < 0.05$) higher on Anza from California than on any of the six cultivars grown in the southeastern United States (Table 3). Reproductive rates on these six cultivars varied, both within and between nematode isolates. *M. incognita*

TABLE 2. Reproduction (eggs/g fresh root) of *Meloidogyne incognita* and *M. javanica* on wild and domesticated wheats.

	Experiment 2				Experiment 3	
		M. incognita†			*M. incognita*	
	M. javanica	Race 1	Race 2	Race 3	Race 1	Race 3
Aegilops squarrosa L. (2n = 14)						
G 1279	3,769 ab	253 ab	412 ab	384 a	3,249 a	2,803 a
G 3489	377 c	15 d	18 d	21 d	40 c	23 c
T. turgidum L. var. *durum* (2n = 28)						
cv. Cocorit	4,578 a	250 ab	204 bc	45 cd	2,779 a	1,987 a
cv. Produra	3,572 ab	621 a	1,241 a	366 ab	2,968 a	830 a
Triticum aestivum L. (2n = 42)						
cv. Anza	2,759 ab	129 b	260 b	227 ab	4,998 a	1,745 a
cv. Chinese Spring	2,176 b	66 bc	117 bc	90 bc	779 b	139 b
Synthetic allohexaploid G 4299 (Produra × G 3489)	388 c	46 cd	64 c	31 cd	46 c	23 bc

Each value is the mean of five replicates.
Means followed by the same letter within a column are not different based on Duncan's multiple-range test ($P < 0.05$) performed on $\log_{10}(x + 1)$ transformed data.
† Three isolates from North Carolina State University Collection.

race 2 produced fewer eggs on Coker 747 and Southern Bell than on other cultivars. *M. incognita* race 3 produced more eggs on Delta Queen and McNair 1813, whereas *M. javanica* produced more eggs on McNair 1813 than on other southeastern United States cultivars. All three root-knot isolates reproduced significantly less ($P < 0.05$) on the synthetic allohexaploid G 4299. *M. incognita* race 2 produced more eggs than *M. incognita* race 3 or *M. javanica* on all the bread wheat genotypes (Table 3). *M. javanica* reproduced poorly on Coker 68-15 and Double Crop.

TABLE 3. Reproduction (eggs/g fresh root) of *Meloidogyne incognita* and *M. javanica* on bread wheat cultivars, *Triticum aestivum* (2n = 2x = 42).

	M. incognita		
	Race 2†	Race 3	*M. javanica*
Anza	NT‡	10,354 a	16,204 a
Coker 747	3,381 c	454 d	1,566 c
Coker 68-15	8,981 ab	416 d	854 c
Delta Queen	21,293 a	1,048 bc	1,173 c
Double Crop	12,790 a	402 d	727 c
McNair 1813	13,476 a	1,321 b	2,993 b
Southern Bell	5,885 bc	658 cd	1,674 c
Synthetic allohexaploid G 4299	422 d	90 e	200 d

Each value is the mean of five replicates.
Means followed by the same letter within a column are not different based on Duncan's multiple-range test ($P < 0.05$) performed on $\log_{10}$ transformed data.
† This isolate from North Carolina State University Collection.
‡ Not tested.

DISCUSSION

Wild relatives of wheat are being exploited for sources of resistance to different pests and pathogens. Resistance to many insect, fungal, and nematode diseases have been identified in wild *Aegilops* species (2–5,9,15). Our results clearly demonstrate that the resistance to *M. incognita* and *M. javanica* derived from the diploid parent *Ae. squarrosa* G 3489 is phenotypically expressed in the synthetic allohexaploid G 4299. The phenotypic expression of resistance indicates lack of significant interference by genomic components contributed by Produra, the root-knot susceptible tetraploid parent. In addition, the onefold genomic contribution of *Ae. squarrosa* (DD) combined with a twofold genomic contribution of susceptible Produra (AABB) implies a dominance of the resistance.

The high level of resistance expressed in the synthetic hexaploid against the several isolates of two *Meloidogyne* species is a valuable character for use in future backcross-

ing with desirable hexaploid types to produce commercial cultivars. In addition to nematode resistance, the parent *Ae. squarrosa* G 3489 and the synthetic allohexaploid G 4299 exhibit increased nitrogen uptake rates, compared with Produra (6).

Our results using isolates representing two host races of *M. incognita* inoculated onto southeastern commercial bread wheat cultivars demonstrated intraspecific variability in *M. incognita* for wheat parasitism. The considerably lower reproductive rates of *M. incognita* race 3 on southeastern wheats compared with that on Anza, the semidwarf Mexican wheat presumably of quite different origin, suggest that modern day commercial hexaploid bread wheats may vary considerably, depending on genomic background. *M. incognita* race 2 reproduced extensively on most of the wheats. This result contrasts with the report of resistance to *M. incognita* in the same commercial bread wheats (1). In these studies Birchfield (1) used a single isolate of *M. incognita* of unspecified race that produced a host reaction in wheat similar to that of the *M. incognita* race 3 isolate. This apparent intraspecific variation in *M. incognita* underscores the caution needed when generalizing, on the basis of studies using a single isolate, about the parasitic ability of a highly variable species such as *M. incognita.*

Isolates of the same race, identified by North Carolina differential host test, differ in their reproductive rates on some wheat cultivars (Table 2). Although some of this variability could be due to conditions extent during different experiments, the response of the cultivars suggest additional within-race variability.

The level of resistance in the southeastern commercial wheats was moderate compared with the highly resistant reactions obtained with *Ae. squarrosa* G 3489 and its synthetic allohexaploid G 4299. This resistance is uniformly effective against a wide range of *M. incognita* and *M. javanica* isolates representing different races from different geographical areas and different field cropping histories, as determined here and in previous experiments (15). The possibility exists, however, that agressive populations of these species may occur that are able to reproduce on wheats whose resistance is based on the DD genome contributed by *Ae. squarrosa* G 3489.

The variability in susceptibility to the root-knot nematodes observed in the commercial bread wheats from the southeastern United States and from California is not surprising. Although wheat is predominantly self pollinated, recurrent hybridization between cultivated polyploid wheats (AABB or AABBDD) and *Ae. squarrosa* (DD) variants may explain how the wide gene pool from *Ae. squarrosa* could be absorbed and considerable heterogeneity introduced (20). This could explain the array of resistant and susceptible hexaploid wheats that occur today. If the DD genome of the hexaploid by chance carried the resistance trait, as in the case of *Ae. squarrosa* G 3489, the result would be a resistant bread wheat. On the other hand, incorporation of the DD genome from the susceptible *Ae. squarrosa* accession G 1279 into polyploid wheat would result in a susceptible bread wheat.

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