RESEARCH/INVESTIGACIÓN

EVALUATING DIVERSE SORGHUM GENOTYPES USED IN BREEDING PROGRAMS FOR RESISTANCE TO *MELOIDOGYNE INCOGNITA*

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ABSTRACT

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The southern root-knot nematode, *Meloidogyne incognita*, is widespread in the tropical, subtropical, and warm temperate areas of the world, where it causes damage to a wide variety of crops. Sorghum (*Sorghum bicolor*) is an important grain and forage crop in many parts of the world, and it is especially important in drier areas where other crops may struggle to produce well. Sorghum is typically a good host for *M. incognita*, although a few resistant genotypes have been reported. Identifying additional sources of resistance could prolong the effectiveness of currently available sources. We evaluated a selection of genetically diverse sorghum genotypes, including several prominent hybrids and inbreds that are used in sorghum breeding programs, in controlled reproduction tests in a greenhouse for resistance to *M. incognita*. None of the genotypes tested were highly resistant, however, five genotypes (BTx378, BTx399, Tx7000, SC1154, and msd2) were moderately resistant. 'Honey Drip' was the resistant standard in the test and was confirmed to be resistant, and the moderately resistant genotypes did not contain a haplotype similar to the 'Honey Drip' resistance QTL region on chromosome 3, and, therefore, likely represent at least one previously undescribed source of resistance.

Key words: Sorghum bicolor, southern root-knot nematode

RESUMEN

Davis, R. F., K. Harris-Shultz, C. Hayes, Z. Xin, y J. E. Knoll. 2024. Evaluación de diversos genotipos de sorgo utilizados en programas de mejoramiento para resistencia a *Meloidogyne incognita*. Nematropica 54:166-176.

El nematodo agallador del sur, *Meloidogyne incognita*, está muy extendido en las zonas tropicales, subtropicales y templadas cálidas del mundo, donde causa daños a una amplia variedad de cultivos. El sorgo (*Sorghum bicolor*) es un importante cultivo de cereales y forrajes en muchas partes del mundo, y es especialmente importante en las zonas más secas donde otros cultivos pueden tener dificultades para producir bien. El sorgo suele ser un buen hospedante para *M. incognita*, aunque se han descrito algunos genotipos resistentes. La identificación de fuentes adicionales de resistencia podría prolongar la eficacia de las fuentes actualmente disponibles. Evaluamos una selección de genotipos de sorgo genéticamente

diversos, incluidos varios híbridos y endogámicos destacados que se utilizan en programas de mejoramiento del sorgo, en pruebas de reproducción controlada en un invernadero para determinar la resistencia a *M. incognita*. Ninguno de los genotipos evaluados fue altamente resistente, sin embargo, cinco genotipos (BTx378, BTx399, Tx7000, SC1154 y msd2) fueron moderadamente resistentes. Se confirmó que 'Honey Drip' era resistente y los genotipos moderadamente resistentes no contenían un haplotipo similar a la región QTL de resistencia de 'Honey Drip' en el cromosoma 3 y, por lo tanto, probablemente representan al menos una fuente de resistencia no descrita previamente.

Palabras clave: *Sorghum bicolor*, nematodo agallador del sur

INTRODUCTION

Sorghum (*Sorghum bicolor*) grows well in semi-arid, tropical areas and is an important grain for both humans and livestock. Because it can be grown on land poorly suited to many crops, it has potential as a bioenergy crop (Jiao *et al.*, 2016). Most of the sorghum grown in the U.S. is produced in drier states including Kansas, Texas, and Oklahoma, but it is well suited to serve as a drought-tolerant rotation crop in the southeastern U.S. Several types of sorghum are grown for different purposes, including grain sorghum, sweet sorghum, and forage sorghum. Although the sorghum types have large phenotypic differences, they are all *S. bicolor* and can easily be crossed to move desirable traits among types through traditional breeding.

Meloidogyne incognita, the southern root-knot nematode, is both widespread geographically and has a very wide host range (Sasser and Carter, 1985), which includes sorghum. *Meloidogyne incognita* is recognized as an important pathogen of grain sorghum (McGawley and Overstreet, 1998), although the reported host status of sorghum hybrids for *M. incognita* has been variable. A selection of 10 hybrids were poor hosts for *M. incognita* and successfully suppressed nematode levels in South Carolina (Fortnum and Currin, 1988). In Florida, two studies by the same authors reported a sorghum hybrid as a poor host for *M. incognita* in one study (McSorley and Gallaher, 1991) and a different hybrid as a good host in another study (McSorley and Gallaher, 1992), which led the authors to conclude that hybrid selection was crucial for managing *M. incognita* (McSorley and Gallaher, 1992). More recently, controlled reproduction studies have also found mixed results for the susceptibility of sorghum, although most hybrids were found to range from moderately susceptible to very susceptible with only a few being resistant (de Brida *et al.*, 2017; Hurd and Faske, 2017; Xavier-Mis *et al.*, 2017). Two quantitative trait loci (QTLs) for resistance to *M. incognita* were identified in sweet sorghum genotypes (Harris-Shultz *et al.*, 2015; Harris-Shultz *et al.*, 2019). However, those resistance QTLs have not yet been moved into sorghum hybrids, although marker-assisted selection was used effectively to move a resistance QTL on chromosome 3 into susceptible genotypes (Davis *et al.*, 2021).

Sorghum is a diploid plant (Jiao *et al.*, 2016) with minimal gene redundancy (Patterson *et al.*, 2009). Hybrid development utilizes male sterile lines (A lines), fertility maintainer lines (B lines), and fertility restorer lines (R lines) (Rakshit and Bellundagi, 2018). Published reports have evaluated selected hybrids for resistance to *M. incognita*, but there has been no evaluation of genotypes commonly used to create hybrids or of germplasm collections to identify resistance sources. Identification of A, B, or R lines with resistance to *M. incognita* would facilitate the development of resistant hybrids. Additionally, if resistance were identified in genotypes previously used in hybrid development, the progeny of those genotypes should be more likely than randomly selected genotypes to also have resistance, which would allow for a more targeted search for additional resistant genotypes. Our goal in this study was to evaluate *M. incognita* reproduction on a diverse selection of sorghum genotypes that included widely grown hybrids and inbreds commonly used in sorghum breeding programs

MATERIALS AND METHODS

Plant phenotyping

The reproduction of *M. incognita* was evaluated on 24 sorghum genotypes (Table 1).

Table 1. Sorghum genotypes evaluated for resistance to *Meloidogyne incognita*.

Name	Notes
BTx623	B line[y], genome sequence available
BTx642	B line, source of stay-green
BTx2752	B line
BTx378	B line
BTx399	B line
RTx436	R line[z]
RTx437	R line
RTx2783	R line, resistant to sugarcane aphids
Tx7000	R line, susceptible to sugarcane aphids, senescent line (no stay-green)
Macia	R line
Rio	Sweet, genome sequence available
Abjabsido	R line
P898012	Dhurrin type, drought tolerant
SC1154	Dhurrin type, stalk rot resistant, source of stay-green
K73-J6	Hybrid, sugarcane aphid susceptible
SP6929	Hybrid
P84G62	Hybrid
ATx642/RTx437	Hybrid
DKS53-67	Hybrid, high yielding, susceptible to sugarcane aphids
msd1	Multi-seeded mutant of BTx623
msd2	Multi-seeded mutant of BTx623
Honey Drip	Sweet; *Meloidogyne incognita*-resistant standard
Collier	Sweet; *Meloidogyne incognita*-susceptible standard
Blade ES 5200	Forage/high biomass hybrid

[y]"B line" indicates a fertility maintainer line

[z]"R line" indicates fertility restoring line

Twenty genetically diverse genotypes were selected and represented important hybrids or inbred lines used in sorghum breeding. All of the hybrids, except ATx642/RTx437, were obtained from commercial sources. Two genotypes (*msd1* and *msd2*, mutants of BTx623 developed by Z. Xin) were selected because they contain mutations that reduce the production of jasmonic acid (Gladman *et al.*, 2019), which is involved in the plant's innate defense system and can affect nematode parasitism (Gheysen and Mitchum, 2019). 'Honey Drip' was included as a resistant standard, and 'Collier' was included as a susceptible standard (Harris-Shultz *et al.* 2015). Two greenhouse tests were conducted, each with 6 replications in a randomized complete block design.

For each test, four seeds of a genotype were planted into a 15-cm-diam. pot containing pasteurized soil (Tifton Loamy Sand). Approximately 10 days after emergence, plants were thinned to 1 plant per pot. Approximately 3 wk after planting, plants were inoculated with *M. incognita*. Pots were inoculated with 8,000 eggs per pot in the first test, and 10,000 eggs per pot in the second test. The *M. incognita* culture used for inoculum was maintained on cotton (*Gossypium hirsutum*) to prevent contamination with other *Meloidogyne* species in the greenhouse and increased on eggplant (*Solanum melongena*) for approximately 3-4 nematode generations prior to use. Eggs for inoculum were collected from eggplant roots by cutting roots into 5-cm-long pieces, agitating roots in 0.5% NaOCl solution for 2 min, collecting the eggs on nested 150- over 25-um-pore sieves, and then rinsing with tap water (Hussey and Barker, 1973). Inoculum was divided into 2, 2.5-cm-deep holes, one on each side of the plant stem. After inoculation, the holes were covered with soil, and the pots were watered.

Eight weeks after inoculation, nematode reproduction and root weight data were collected. All roots recovered from each pot were rinsed thoroughly to remove as much soil as possible, and then roots were gently dried with paper towels, and fresh root weights were determined. Nematode eggs were extracted from all sorghum roots recovered from each pot by rinsing roots free of

soil, cutting roots into 5-cm-long pieces, agitating roots in 1% NaOCl for 4 min, and collecting eggs as previously described. Nematode eggs were counted from a subsample for each pot, and the total number of eggs per pot was calculated. The number of eggs per gram of fresh root weight was calculated.

The number of eggs per pot (total eggs) and the number of eggs per gram of root (eggs/g root) were analyzed using the GLIMMIX procedure in SAS. Total eggs and eggs/g root were $\log_{10}$ transformed prior to analysis; however, all data is presented as arithmetic means. An initial analysis was performed with combined data from the two tests, but there was a significant test×genotype interaction for both total eggs and eggs/g root, which indicates that the effect of genotype was not consistent between trials, so the two tests were analyzed separately. Mean separations were performed with the LSMEANS (least squares means) statement, which calculates the least squares means and compares them via multiple t-tests. All tests for statistical significance are at α=0.05 unless stated otherwise.

Plant genotyping

Leaf tissue of BTx623, BTx642, BTx2752, BTx378, BTx399, RTx436, RTx437, RTx2783, Tx7000, Macia, Rio, Abjabsido, P898012, SC1154, K73-J6, SP6929, ATx642/RTx437, DKS53-67, *msd1*, *msd2*, 'Honey Drip' (resistant standard), 'Collier' (susceptible standard), Blade, and P84G62 (Table 1) was ground to a fine powder by placing tissue into 2-ml tubes containing 4 sterilized zinc-plated BBs (Daisy Outdoor Products, Rogers, AR). These tubes were then placed in liquid nitrogen, and ground on a vortexer for less than 5 sec before repeatedly being placed back in liquid nitrogen after grinding so that the sample always remained frozen. DNA was extracted using a GeneJET Plant Genomic DNA Purification Mini Kit (Thermo Fisher Scientific, Waltham, MA). Simple sequence repeat (SSR) markers (Table 2) were amplified for each sample using a 10 µL reaction containing 2 µL of 5x Colorless GoTaq Flexi buffer, 1 µL of 25 mM MgCl2, 0.8 µL of 2.5 mM dNTPs, 1.8 µL of M13-800 (M13-TGTAAAACGACGGCCAGT), 0.4 µL of GoTaq Flexi DNA Polymerase, 0.86 µL of water, 0.5 µL of forward primer (1 µM) where the 5'end of the primer was M13 tagged, 2 µL of reverse primer (1 µM), and 1 µL of DNA (2.5 ng/µL). Two water controls (not containing DNA) were included per marker reaction. The thermocycler conditions were initial denaturation at 94°C for 3 min, 39 cycles of 94°C for 30 sec, 50 °C for 1 min, 72°C for 1 min and 10 sec, and a final elongation step at 72°C for 10 min. Two microliters of PCR product were combined with 5 µL of BlueStop (LI-COR Biosciences, Lincoln, NE) and 0.35 µL of this mixture was loaded onto a 6.5% acrylamide gel using a LI-COR Biosciences 4300 DNA Analyzer. Gel images were scored visually and coded as a "1" for the presence of a band, "0" for the absence of a band, or "9" for missing (failed reaction) for each accession for each fragment. For the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis, the binary marker data matrix was imported into NTSYSpc (Rohlf, 2008). Genetic similarity between each pair of samples was calculated using the SIMQUAL module using the DICE coefficient of similarity (Nei and Li, 1979). A dendrogram was generated from the similarity matrix by using the UPGMA procedure in the SAHN module of NTSYSpc. The software program FreeTree (Hampl *et al.*, 2001) was used to conduct the bootstrapping analysis, and the number of repetitions was 1000. Only bootstrap values ≥50% are shown.

RESULTS

All comparisons for statistical differences are at α=0.05 unless otherwise stated. *Meloidogyne incognita* reproduction as measured by total egg production was much greater in the first test than the second test. In the first trial, *M. incognita* produced fewer eggs on the resistant standard, 'Honey Drip', than on any other genotype (Table 3). Significant differences in total eggs were found among the remaining genotypes, with mean total eggs (excluding 'Honey Drip') ranging from 57,300 to 685,350. However, only DKS53-67 was statistically different from the susceptible standard, 'Collier', with DKS53-67 having more eggs than 'Collier'. The mutant msd1 did not differ from *msd2* or BTx623, but *msd2* had fewer total eggs than BTx623. The reproduction factor (RF = Final Population [Pf]/Initial Population [Pi]) was 0.8 for the resistant standard, 26.1 for the susceptible standard, and the RF ranged from 7.2 to 85.7 for the other genotypes.

Table 2. A list of simple sequence repeat markers and their characteristics that were used to assess genetic diversity throughout the sorghum genome or only in the Honey Drip QTL region.

Marker	Region	Chromosome	Forward sequence	Reverse sequence	Motif	Annealing Temperature Used (°C)	Expected Amplicon (bp)[z]	Reference
Cup07	Genome	10	CTAGAGGATTGCTGGAAGCG	CTGCTCTGCTTGTCGTTGAG	(CAA)8	50	251	Schloss *et al.* 2002
Cup16	Genome	10	TGCAGTGCTAGCTCATGGTC	CTTTCCAGCCTCCCATATCC	(CTTTT) 4	50	222	Schloss *et al.* 2002
Cup43	Genome	10	GCCTAACTCCCTTGTGATGC	GTCAGTGGATGTGGATGTGC	(CTGCC)5	50	249	Schloss *et al.* 2002
RKNP135	Honey Drip QTL region	3	GTTTCGTTTCAATCGGCTTC	GCGCCCCATCATATGTCTT	(AAG)21	50	197	Harris-Shultz *et al*. 2015
RKNP342	Honey Drip QTL region	3	TTCCAACAGGCAAACAACAG	TCATGGCCTGTGTATCAAGC	(CT)25	50	205	Davis *et al*. 2021
RKNP402	Honey Drip QTL region	3	TCAGCAAGATGGTTGGTTGA	ACGAGGCCGTTGAGATTATG	(TTA)22	50	213	Davis *et al*. 2021
txp21	Genome	4	GAGCTGCCATAGATTTGGTCG	ACC TCG TCC CAC CTT TGT TG	(AG)18	50	179	Kong *et al*. 2000
txp265	Genome	6	GTCTACAGGCGTGCAAATAAAA	TTACCATGCTACCCCTAAAAGTGG	(GAA)19	50	209	Bhattramakki *et al*. 2000
txp40	Genome	7	CAGCAACTTGCACTTGTC	GGGAGCAATTTGGCACTAG	(GGA)7	50	138	Kong *et al*. 2000
txp496	Genome	3	TCCACAACCTTGCTCTTCCT	CATGGCGGTCTAGCTACCTC	(CTT)16	50	69	Zhou, 2006
txp516	Genome	8	TCCGAGACAACAGGGAGAAG	TCCCGCTACTGCATTTCTTT	(AG)14	50	172	Davis *et al*. 2024 (This study).
txp57	Genome	6	GGAACTTTTGACGGGTAGTGC	CGATCGTGATGTCCCAATC	(GT)21	50	249	Bhattramakki *et al*. 2000
txp94	Genome	5	TTTCACAGTCTGCTCTCTG	AGGAGAGTTGTTCGTTA	(TC)16	50	232	Bhattramakki *et al*. 2000
txp97	Genome	6	CAAATAAACGGTGCACACTCA	GTATGATTGGAGACGAGACGG	(CA)8, (CGC)6	50	129	Bhattramakki *et al*. 2000

[z]Expected amplicon size was obtained from the genomic sequence of sorghum genotype BTx623.

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Table 3. Reproduction of *Meloidogyne incognita* on select sorghum genotypes in a greenhouse (Trial 1).

Genotype	Eggs[z]	Eggs/g root[z]
BTx623	100,350 fg	2,639 fghi
BTx642	269,400 bc	5,769 defg
BTx2752	217,350 bcde	9,243 abcde
BTx378	103,650 fg	3,231 ij
BTx399	151,350 bcdefg	3,919 fghi
RTx436	169,950 bcdefg	5,986 abcd
RTx437	172,200 bcdef	5,089 abcd
RTx2783	186,120 bcdef	6,877 ab
Tx7000	57,300 g	3,030 j
Macia	202,950 bcde	4,033 bcde
Rio	148,050 cdefg	1,764 a
Abjabsido	429,000 ab	10,360 abc
P898012	78,300 efg	1,075 hij
SC1154	100,800 efg	4,324 fghi
K73-J6	232,200 bcd	8,072 cdefg
SP6929	447,300 ab	14,877 a
P84G62	307,125 ab	10,482 cdefg
ATx642/RTx437	407,850 ab	8,358 defg
DKS53-67	685,350 a	18,753 a
msd1	175,680 bcdef	6,567 efgh
msd2	108,000 bcdefg	2,914 hij
Honey Drip	6,300 h	126 k
Collier	208,980 bcdefg	5,047 abcde
Blade ES 5200	92,340 defg	1,342 ghij

[z]Means followed by the same letter are not different at the $\alpha = 0.05$ significance level.

'Honey Drip' supported the fewest eggs/g root in the first test, with a mean of 126 eggs/g root, which was statistically lower than any other genotype (Table 2). Eggs/g root for the genotypes other than 'Honey Drip' ranged from 1,075 to 18,753. In addition to the resistant control, seven genotypes had statistically fewer eggs/g root than the susceptible control, 'Collier', and no genotypes had statistically more eggs/g root than 'Collier'. The mutants *msd1* and *msd2* did not differ from BTx623 or from each other.

In the second test, which had much lower nematode reproduction than the first test, 300 total eggs were produced on 'Honey Drip', and 3,250 to 138,033 eggs were produced on the other genotypes (Table 4). 'Honey Drip' had statistically fewer total eggs than any other genotype. Collier had 48,550 eggs, and six genotypes in addition to 'Honey Drip' had statistically fewer eggs than Collier, but no genotypes had statistically more eggs than 'Collier'. The mutant msd1 did not differ from BTx623, but *msd2* had fewer total eggs than BTx623; however, *msd1* and *msd2* did not differ from each other. The RF value for 'Honey Drip' was 0.03, the RF for 'Collier' was 4.9, and RF values for the other genotypes ranged from 0.3 to 13.8.

In the second test, 'Honey Drip' had 8 eggs/g root, which was significantly fewer than any other genotype (Table 3). Genotypes other than 'Honey Drip' ranged from 179 to 3,242 eggs/g root. Eight genotypes had significantly fewer eggs/g root than 'Collier', and no genotypes had statistically more eggs/g root than 'Collier'. The mutants *msd1* and *msd2* did not differ from BTx623.

Genotyping of the 24 sorghum genotypes with three polymorphic SSR markers within the 'Honey Drip' QTL region (Harris-Shultz *et al.* 2015) generated 26 fragments (Fig. 1). Three groups were formed, and 'Honey Drip' had a region that was not similar to any of the other lines tested. Genotyping of the same lines with 11 polymorphic SSR markers located on multiple chromosomes generated 42 fragments, and three groups were also formed (Fig. 2). Yet, using these markers 'Honey Drip' was similar to another sweet sorghum line, 'Collier', which is highly susceptible to *M. incognita* (Harris-Shultz *et al.* 2015).

Table 4. Reproduction of *Meloidogyne incognita* on select sorghum genotypes in a greenhouse (Trial 2).

Genotype	Eggs[z]	Eggs/g root[z]
BTx623	26,599 def	555 fghi
BTx642	56,960 abcde	857 defg
BTx2752	52,826 abcde	1,463 abcde
BTx378	5,050 gh	235 ij
BTx399	13,828 fgh	410 fghi
RTx436	39,950 abcde	1,517 abcd
RTx437	57,400 abcd	1,932 abcd
RTx2783	111,000 ab	2,464 ab
Tx7000	3,250 h	187 j
Macia	68,162 abcd	1,053 bcde
Rio	138,033 a	2,730 a
Abjabsido	50,600 abcde	1,929 abc
P898012	16,359 efg	266 hij
SC1154	10,150 fgh	433 fghi
K73-J6	29,592 bcde	895 cdefg
SP6929	95,200 abc	3,242 a
P84G62	27,200 cde	805 cdefg
ATx642/RTx437	58,598 abcde	1,008 defg
DKS53-67	107,400 ab	3,061 a
msd1	18,643 fgh	407 efgh
msd2	9,140 gh	179 hij
Honey Drip	300 i	8 k
Collier	48,550 abcde	1,387 abcde
Blade ES 5200	35,841 bcde	462 ghij

[z]Means followed by the same letter are not different at the $\alpha = 0.05$ significance level.

DISCUSSION

Nematode resistance may be considered high or moderate, depending on the level of suppression compared to a susceptible standard (Hussey and Janssen, 2002). Genotypes that allow less than 10% of the reproduction on a susceptible standard are typically labelled highly resistant, whereas genotypes that allow statistically less reproduction than the susceptible standard but more than 10% of the standard are termed moderately or partially resistant (Hussey and Janssen, 2002). No genotypes in our test, other than 'Honey Drip', expressed a high level of resistance, although Tx7000 appeared highly resistant in the second test but did not differ from 'Collier' in the first test. Although no genotypes were as resistant to *M. incognita* as 'Honey Drip', several genotypes could be considered moderately resistant. Six genotypes (BTx378, BTx399, Tx7000, SC1154, msd1, and msd2) expressed moderate resistance in the second test, but not in the first test; however, the genotypes expressing moderate resistance in the second test all supported numerically (but not statistically) fewer nematodes than the susceptible standard in the first test, which may suggest some level of consistency.

When nematode reproduction is measured on genotypes that have widely varying levels of root mass, nematode levels are sometimes standardized as eggs/g root because the size of the root system can affect the plant's nematode population carrying capacity, which may affect the plant's apparent level of resistance. Six genotypes (BTx378, BTx399, Tx7000, P898012, SC1154, and *msd2*) had fewer eggs/g root in both the first and second tests and would be classified as moderately resistant. In our test, five of the genotypes classified as moderately resistant based on eggs/g root were among those that expressed moderate resistance based on total eggs produced in the second test and had numerically fewer eggs than the susceptible standard in the first test. Based on that relative consistency, we conclude that BTx378, BTx399, Tx7000, SC1154, and *msd2* express moderate resistance, and P898012 may also be moderately resistant.

Resistance is related to the ability of a plant to

affect nematode reproduction, whereas tolerance is related to the degree of damage a nematode inflicts on the plant (Cook and Evans 1987). In addition to providing a way to measure resistance, eggs/g root also provides a measure of the parasitic load on a plant, and the parasitic load may be reflected in the level of tolerance a plant exhibits (Davis and Stetina, 2016). The genotypes in our test with fewer eggs/g root than the susceptible standard may be more tolerant of *M. incognita* parasitism than genotypes with higher levels of eggs/g root. Although sorghum often does not suffer significant yield reductions because of *M. incognita* (Sharma and McDonald, 1990; Koenning *et al.*, 1999), yield loss has been reported (Orr, 1967; Thomas and Murray, 1987; Babatola and Idowu, 1990). Although not tested in our study, incorporating moderate resistance that reduces the parasitic load on a plant could reduce the possibility of yield loss to *M. incognita*.

Genotyping of the 24 sorghum genotypes revealed that 'Honey Drip' likely has a unique haplotype in the 'Honey Drip' QTL region, suggesting only this line has the root-knot nematode resistance region. Our phenotyping data supported this as no other line tested had the level of resistance of 'Honey Drip' (Tables 2 and 3). In a previous study, the root-knot nematode resistant varieties 'Colman' and 'Leoti Peltier' had similarity to 'Honey Drip' in the QTL region (Harris-Shultz *et al.*, 2019).

Genotypes *msd1* and *msd2* are multi-seeded mutants that were derived by applying ethyl methanesulfonate (EMS), a chemical mutagen, to BTx623, resulting in plants that produce twice the number of seeds per panicle (Jiao *et al.*, 2016). The mutations in both msd1 and msd2 reduce the plant's jasmonic acid (JA) production during panicle formation relative to BTx623 (Jiao *et al.*, 2018; Block *et al.*, 2020); however, JA production has not been quantified at other developmental stages. Jasmonic acid is involved in an innate plant defense pathway that can decrease a plant's susceptibility to nematodes (Gheysen and Mitchum, 2019). We hypothesized that if JA suppresses *M. incognita* reproduction in sorghum, then mutants, such as *msd1* and *msd2* that produce less JA, might be more susceptible than their parent, BTx623. However, our data suggest that the mutations had no effect. Although msd2 consistently expressed moderate resistance (relative to the susceptible standard, 'Collier') whereas BTx623 and msd1 did not, our tests showed that *msd1* and *msd2* did not differ from BTx623 in the total number of eggs or in eggs/g root, so we are reluctant to draw any conclusions regarding the effect of the mutations in *msd2* on nematode reproduction. We do not know why

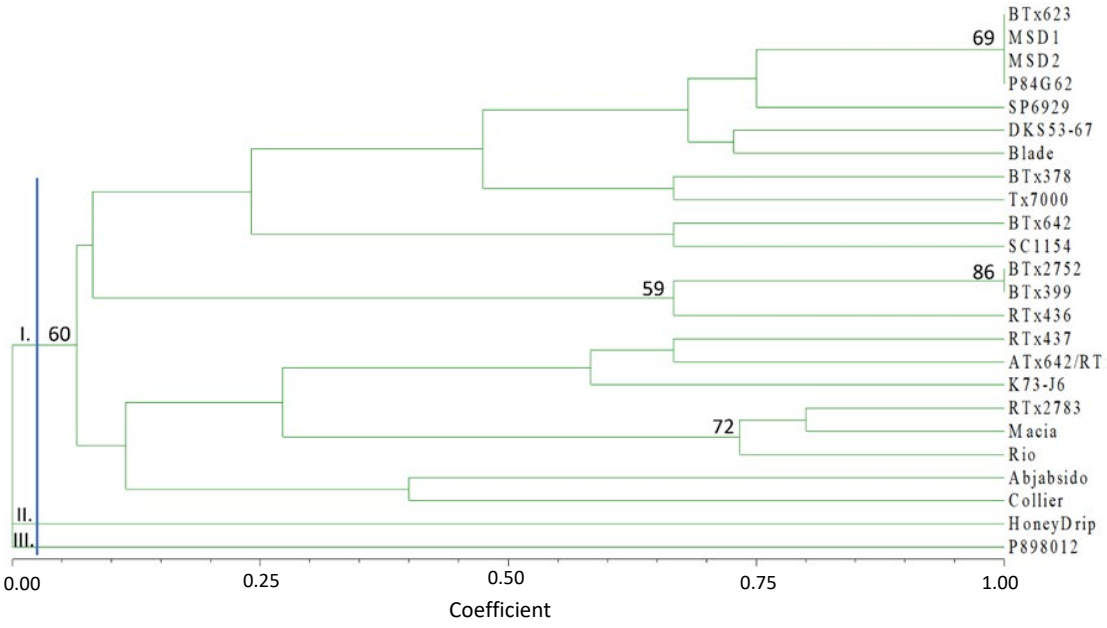

Figure 1. Genotyping of 24 *Sorghum bicolor* genotypes using three polymorphic simple sequence repeat markers within the Honey Drip QTL region. (ATx642/RT= ATx642/RTx437)

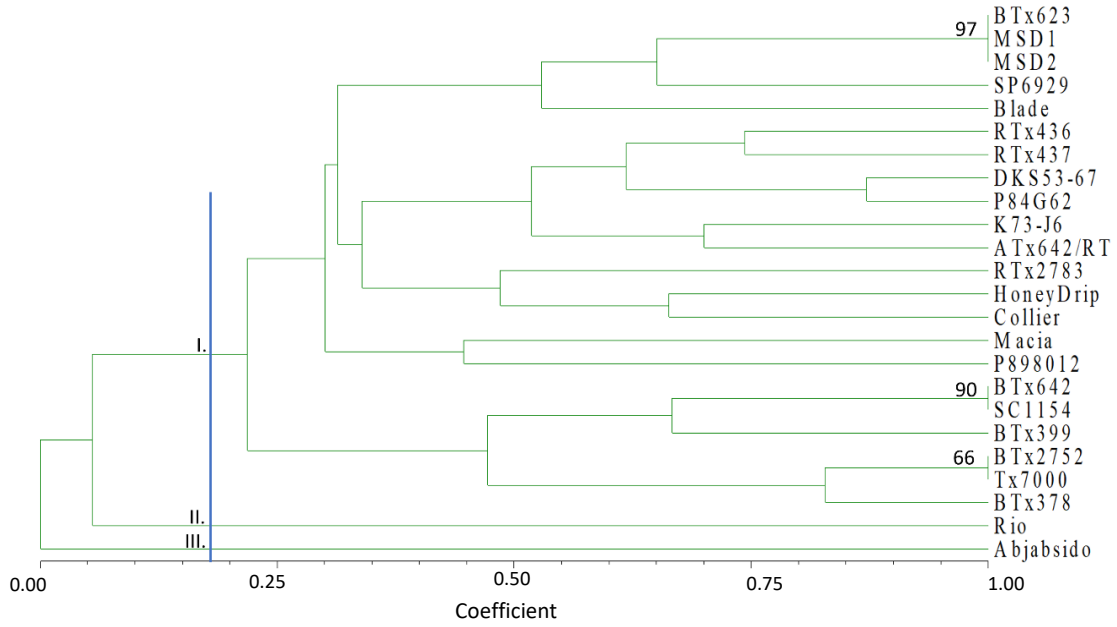

Figure 2. Genotyping of 24 *Sorghum bicolor* genotypes with 11 polymorphic simple sequence repeat markers that are located on multiple chromosomes. (ATx642/RT= ATx642/RTx437)

msd2 expressed moderate resistance and msd1 and BTx623 did not. There are several possible reasons why JA levels may not have had an effect in our study: perhaps JA levels or differences in JA levels were not large enough to have an effect (BTx623 did not express resistance, so reducing JA levels in *msd1* and *msd2* may have had no effect), perhaps differences in JA levels did not occur at times critical to nematode reproduction or development (differences in JA have only been measured during panicle development), perhaps JA has little effect on nematode reproduction in sorghum, or perhaps differences in JA levels occurred only in above-ground tissue and not in root tissue where it would be more likely to affect nematodes.

In this study, we identified several genotypes that had moderate resistance and possibly increased tolerance; however, none had high levels of resistance. Although moderate levels of resistance can be useful (Davis and Kemerait, 2009), moderate resistance is typically not included in breeding efforts when a source with high level of resistance is available, as it is in sorghum (Harris-Shultz *et al.*, 2015; Harris-Shultz *et al.*, 2019). Additional evaluation of the sorghum germplasm collection will be necessary to identify new sources of resistance that impart a high level of resistance via previously unidentified genes. Phenotyping the Sorghum Association Panel (SAP), which is genetically diverse (Casa *et al.*, 2008), may identify additional highly resistant genotypes that have resistance genes different from those previously identified. Having additional sources of resistance would allow for the pyramiding of multiple resistance genes, which should help prolong the usefulness of resistance sources by reducing selection pressure on the nematodes to overcome the plant's resistance.

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