academicJournals

Vol. 12(16), pp. 1411-1414, 20 April, 2017
DOI: 10.5897/AJAR2017.12169
Article Number: 7E0550F63889
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Evaluation of root-knot nematode resistance in sweetpotato

Gregory C. Bernard[1*], Marceline Egnin[1], Conrad Bonsi[2], Desmond Mortley[2], William H. Witola[3], Wendell McElhenney[4] Steven Samuels[1] Caroline Land[5] and Kathy Lawrence[6]

[1]Plant Molecular and Cellular Genetics Lab, Tuskegee University, Tuskegee AL 36088, USA.
[2]George Washington Carver Agricultural Experiment Station, Tuskegee University, Tuskegee, AL 36088, USA.
[3]Parasitology Department, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Chicago, Illinois 61801, USA.
[4]Biostatistics, Department of Agriculture and Environmental Science, Tuskegee University, Tuskegee AL 36088, USA.
[5]Department Plant Pathology, University of Florida, Gainesville, FL 32611, USA.
[6]Department of Entomology and Plant Pathology, Auburn University, AL 36849, USA.

Received 25 January, 2016; Accepted 13 March, 2017

Five healthy, vigorous cultivars of sweet potato [*Ipomoea batatas* (L.) Lam] were cultivated under root-knot nematode *Meloidogyne incognita* challenge to distinguish levels of resistance to infection. Roots and soil samples were collected 84 days' post-infection and evaluated for specific host responses to nematode infection by visual screening analysis and quantitative assessments of symptoms of infection. Resistant control cultivar Nugget showed the highest degree of resistance manifested in lower necrosis and galling, high fresh root weights and low nematode and egg counts. Necrosis and galling scores were highest in susceptible cultivars Georgia Jet and DMO1 where extensive root cracking was observed in Georgia Jet, and storage root development was severely restricted in DMO1 which also produced the highest egg counts. TUO2 and Whatley Loretan were considered intermediately resistant based on egg counts and necrosis and galling. Our results suggest that genotypic differences between cultivars were apparent in multiple host responses to root-knot nematode infection. We have also provided initial evidence to support the identification of newly developed sweet potato cultivars with intermediate resistance to root-knot nematodes.

Key words: Plant-parasitic nematodes, host plant resistance, *Ipomoea batatas*, *Meloidogyne incognita*

INTRODUCTION

Plant-parasitic nematodes cause devastating effects on important crops throughout the world. Root-knot nematodes (RKNs) are a group of well-documented parasites of a wide variety of crops and are considered economically important plant-parasitic nematodes (Calderón-Urrea et al., 2016; Moens and Perry, 2009).

*Corresponding author. Email: gbernard4673@mytu.tuskegee.edu Fax: 334-724-4451.

Table 1. Root necrosis, root galling, and egg and nematode soil counts produced by *Meloidogyne incognita* race 3 and fresh root weights from sweet potato cultivars.

Cultivar	Necrosis and galling	Egg counts	Fresh root weights	Nematode soil counts
Nugget	2c[z]	213[b]	18[a]	31[c]
Ga. Jet	4[ab]	346[b]	15[a]	101[bc]
TUO2	3[ab]	298[ab]	14[ab]	2065[a]
WLA	3[b]	306[b]	9[bc]	134[b]
DMO1	4[a]	481[a]	7[c]	386[ab]

[v]Necrosis and galling values are the mean of five replications of five plants each. Significant differences exist between all of the treated cultivars in this study (P≤0.0001). It is expressed as the percentage of necrotic and galled root tissue. 0= no galling or necrosis, 1 = 1-10% galling; 2 = 11-25% galling and necrosis; 3 = 26-50% galling and necrosis; 4 = 51-75% galling; and 5 = above 75% galling (1-highly resistant 2-resistant, 3-intermediately resistant, 4 -susceptible, 5-highly susceptible. [w]Egg counts/g *inplanta* are the mean of five replications of five plants each. It is expressed as the number of eggs found per gram of root tissue. Significant differences exist between all the treated cultivars in this study (P≤0.01). [x]Fresh root weights are the mean of five replications of five plants each. It is expressed as the total fresh root weights in grams for all five sweet potato cultivars subjected to the inoculation of root-knot nematodes. Significant differences exist between all of the treated cultivars in this study (P≤0.0003). [y]Nematode soil counts are the mean of five replications of five plants each. It is expressed as number of nematode larvae per 100 grams of infected soil from each of the cultivars. Significant differences existed between cultivars in nematode counts from soil samples collected from pots (P≤0.0033). [z]Values within a column followed by the same letter are not different (Fisher's protected LSD test (@=0.05). Transformation of the data used for corresponding ANOVA was log (x+1). Back-transformed data are presented in the tabular format.

When RKN infect sweet potatoes, symptoms are demonstrated as round, spindle-shaped galls and definitive cracks and necrosis in tubers resulting in severe yield losses of 10.2% and reduced marketability of storage roots (Suzuki et al., 2012; Nicol et al., 2011; Overstreet, 2009; Sasser and Frekman, 1987; Bonsi and Phills, 1979). Primary evidence of RKN resistance in sweet potato is often measured through assessments of root-gall indices, root-necrosis, and counts of eggs and egg masses (Piedra-Buena et al., 2011; Cervantes-Flores et al., 2002; Bonsi and Phills, 1979). To increase efficiency in the characterizations of resistant genotypes, primary phenotypic screening analyses should include additional assessments of physiological responses to nematode infection. The development of successful RKN resistant sweet potato breeding programs is dependent on the identification of new RKN resistant sweet potato cultivars (Cervantes et al., 2002; Piedra-Buena et al., 2011). The conserved use of existing resistant cultivars may contribute to increased pathogen aggressiveness resulting in epiphytotic conditions. In the southern U.S, three sweet potato cultivars were developed to be included into sweet potato breeding programs however; the levels of RKN resistance in each breeding line has not been characterized. The objective of this study was to distinguish these sweet potato cultivars for resistance to RKNs.

MATERIALS AND METHODS

Five sweet potato cultivars (Nugget, Georgia Jet, TU-02, Whatley Loretan, and DMO1) were evaluated for resistance or susceptibility to RKN infection. Nugget (Cervantes-Flores et al., 2002) and Georgia Jet (Overstreet, 2009) demonstrate unique host responses to M. incognita; and served as resistant and susceptible controls. 12-cm long cuttings were excised from healthy, vigorous sweet potato vines and allowed to root in tap water for of 4 days, and transplanted into 500 cm^3 Styrofoam food containers (Dart Container Co.® Mason, MI) containing a sterilized media of 4-parts coarse sand and 1-part field soil (88.9% sand, 8.3% silt 2.8% clay). *Meloidogyne incognita* race 3 was previously identified using the NC Differential Host test (Hartman and Sasser, 1985) and perineal pattern analysis (Taylor and Netscher, 1974). Nematode inoculum was cultured on susceptible peanut (*Arachis hypogea* L.) plants. RKN eggs were extracted from peanut roots using Hussey and Barker's (1973) NaOCL extraction technique and approximately 10,000 eggs were injected into the soil of each plant at day 14 post-planting for infestation. Inoculated plants and untreated controls were grown under controlled greenhouse conditions of 25-28°C, 16 h light and watered daily. Plants were harvested 84 Days after inoculation and fibrous and storage roots were visually rated for the percentages of total root system that were galled and necrotic based on the visual screening method developed by Bonsi (1982). Root samples were assigned index values using a 1-5 root galling and necrosis index similar to the root gall index previously described and also by Kinloch et al. (1987), where 1 represented highly resistant (0% no galling or necrosis) 2: resistant (2-25% galling and necrosis,) 3: intermediate resistant (26-50% galling and necrosis) 4: susceptible (over 50% galled and necrotic) 5: highly susceptible (over 75% galled, with larger than average size galls and severe necrosis). Fresh root weights were recorded at harvest. Nematode eggs were extracted from a maximum of 3 g of each root system and enumerated under an inverted microscope to estimate total eggs per gram of root system based on root fresh weights. Nematode larvae were obtained from 100 cc of soil obtained from treated plants using the Baermann funnel method (Baermann, 1917) and counted under an inverted microscope. All data were transformed by log (x+1) to standardize the variance and subjected to analysis of variance (ANOVA) using the SAS GLM Procedure (SAS Institute, Cary, N.C.). Treated and untreated samples were arranged in a completely randomized design with five replications per genotype. Treatment means were compared using Fisher's LSD procedure (α=0.05). Back transformed means are presented in tabular format in Table 1 for clarity.

RESULTS

There were significant differences among cultivars for necrosis and galling index scores (P <0.0001), nematode counts (P<0.0033) and root fresh weights (P<0.0003) and statistical differences in egg counts (P<0.01) (Table 1). The number of nematode eggs produced per gram of root tissue was lowest in Nugget in comparison to all cultivars. In comparison to Nugget, Georgia Jet, TUO2, WLA and DMO1 showed increased egg counts of 62.4, 39.9, 43.6 and 125.8%, respectively. While the roots of Nugget showed low galling and necrosis, moderate galling and less necrosis were shown in WLA and TUO2 roots in comparison to the susceptible cultivars which is indicative of a measurable degree of intermediate resistance. The highest incidence of necrosis and galling were shown in the susceptible control Georgia Jet and DMO1. Fresh root weights were highest in Nugget and Georgia Jet. In comparison to Nugget, mean root weight percentages decreased by 16.6, 22.2, 50 and 61.1% in Georgia Jet, TUO2, WLA and DMO1, respectively. Soil nematode counts were lowest for Nugget similar to that of Georgia Jet, while TUO2 had the highest. An increase in nematode soil counts of 225.8, 6561.2, 332.2 and 1145% was shown in Georgia Jet, TUO2, WLA and DMO1 respectively, in comparison to Nugget.

DISCUSSION

Resistance to RKN infection is manifested as a decrease or inhibition of nematode reproduction (Trudgill, 1991; Corbett et al., 2010) or prevention of feeding site establishment (Williamson and Kumar, 2006; Corbett et al., 2010). Although not significantly different from susceptible control cultivar Georgia Jet, Nugget was significantly different from DMO1 (P<0.01) which produced the highest egg counts per gram of root tissue and was considered the most susceptible cultivar, followed by susceptible control Georgia Jet. Decreased egg counts per gram of root tissue was shown in TU02 and WLA when compared to susceptible control Georgia Jet and DMO1 which suggested resistance. Reductions in the severity of root galling and necrosis is a primary attribute of resistance in sweet potato genotypes (Bonsi and Phills, 1979; Cervantes-Flores et al., 2002; Piedra-Buena et al., 2011). Root samples from DMO1 were severely necrotic with minor storage root development in comparison to Georgia Jet and others and a 2.5-fold decrease in root weights occurred between Nugget and DMO1. The ability to tolerate pathogen infection with minor effects on agronomic performance such as yield has been shown in earlier studies and may be attributed to genotypic specificities in resistance. Plant responses to biotic factors may be associated with alterations in root growth. Corbett et al. (2011) suggested increased root weights in resistant tomato genotypes when compared to un-inoculated resistant plants, to be associated with RKN challenge and further indicated that resistant plants displayed a measure of tolerance. A similar response was shown in resistant control Nugget, where root fresh weights were higher in treated plants compared to controls. Though not significant, there was a 31.3% difference in mean nematode counts between Nugget and Georgia Jet. Secondary metabolites produced by different plant species have shown antagonistic effects against plant parasites (Stahl et al., 2016; Mazid et al., 2011). Additional research is needed to identify any potential differences in secondary metabolite production between these genotypes which may correlate to decreased nematode soil numbers.

The evidence presented in this study strongly suggested a correlation between the amplitude of resistance and genotype specificity during the evaluation of multiple host responses to sweet potato cultivars under RKN burden. The resultant data from this analysis confirmed previous evidence of resistance and susceptibility to RKN in existing control varieties and provided new information on host-nematode reactions in three newly developed sweet potato cultivars. To fully assess differential expression patterns among different genotypes, a comprehensive evaluation of expressed transcripts of sweet potato roots under RKN challenge is currently underway in our laboratory. The identification of molecular pathways involved in the host responses, and the development of genetic markers for resistance will provide efficiency in successful RKN resistant sweet potato breeding programs and contribute to the limited genetic information of the discreet processes in sweet potato physiological development under biotic stress.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank NIFA Evans-Allens, United States Department of Agriculture, National Institute of Food and Agriculture, Tuskegee University, George Washington Carver Experiment Station, Wheat TCAP, Plant Cellular and Molecular Genetics Lab, Tuskegee University for their respective contributions toward the success of the study.

REFERENCES

Baermann G (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) larven in Erdproben. Geneeskundig Tijdschrift voor Nederlandsch Indië 57:131-137.

Bonsi CK (1982). Influence of plant resistance and nematicides on growth and yield of tomato (Lycopersicon esculentum) and on population dynamics of root-knot nematodes (*Meloidogyne incognita*) and (*Meloidogyne* hapla) with an associated study of root-knot nemaotde resistance in lettuce (Lactuca species), Ithaca, New York:

Cornell University, Ph.D. Dissertation.
Bonsi CK, Phills BR (1979). Reaction of twelve sweet potato cultivars and breeding lines to two root-knot species with three experimental methods. HortScience 14:539-541.
Calderón-Urrea A, Vanholme B, Vangestel S, Saben K, Bahaji A, Pha K, Garcia M, Snider A, Gheysen G (2016). Early development of the root-knot nematode *Meloidogyne incognita*. BMC Dev. Biol. doi: 10.1186/s12861-016-0109-x
Cervantes-Flores JC, Yencho GC, Davis EL (2002). Host reactions of sweet potato genotypes to root-knot nematodes and variation in virulence of *Meloidogyne incognita* populations. HortScience 37:1112-1116.
Corbett B, Jia L, Sayler R, Arevalo-Soliz LM, Goggin F (2011). The Effects of Root-Knot Nematode Infection and Mi-mediated Nematode Resistance in Tomato on Plant Fitness. J. Nematol. 43:82-89
Hartman KM, Sasser JN (1985). Identification of *Meloidogyne* species on the basis of differential host tests and perineal-pattern morphology. In: K.R. Barker, C.C. Carter, and J.N. Sasser (Eds.) An advanced treatise on *Meloidogyne*. Vol II: Methodology, NCSU Graphics, Raleigh, N.C.
Hussey RS, Barker KR (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Dis. Report 57:1025-1028.
Kinloch RA, Hiebsch CK, Peacock HA (1987). Galling and yields of soybean cultivars grown in *Meloidogyne* arenaria-infested soil. J. Nematol. 19:233-239.
Mazid M, Khan TA, Mohammad F (2011). Role of secondary metabolites in defense mechanisms of plants. Biol. Med. 3:232-249.
Moens M, Perry R, Starr J, (2009). *Meloidogyne* species- a diverse group of novel and important plant parasites. In: R. N. Perry, M. Moens and J. L. Starr, (Eds). Root-knot nematodes. Wallingford, UK: CAB Int. pp. 1-17.
Nicol JM, Turner SJ, Coyne DL, den Nijs L, Hockland TS (2011). Current nematode threats to world agriculture. In Jones, J., Gheysen, G., Fenoll, C. (Eds), Genomics and Molecular Genetics of Plant-Nematode Interactions. Heidelberg, Germany: Springer Sci. pp. 21-43.
Overstreet C (2009). Chapter 9: Nematodes. In: Loebenstein, G., and Thottappilly, G.(Eds.). The Sweet potato. Berlin, Germany: Springer Sci. pp. 135-159
Piedra-Buena A, López-Pérez JA, Díez-Rojo MÁ, Robertson L, Castro-Lizazo I, Bello A (2011). Screening of three sweet potato (*Ipomoea batatas* L.) cultivars for resistance to different virulence groups of root-knot nematodes (*Meloidogyne* spp.) under controlled conditions. Crop Prot. 30:134-140.
Sasser JN, Freckman DW (1987). A world prospective on nematology: the role of the society. In J. A. Veech and D. W. Dickson (Eds.),Vistas on Nematology, Society of Nematologists, Elsevier Sci. pp. 7-14
Stahl E, Bellwon P, Huber S, Schlaeppi K, Bernsdorff F, Vallat-Michel A, Mauch F, Zeier J (2016). Regulatory and Functional Aspects of Indolic Metabolism in Plant Systemic Acquired Resistance. Molec. Plant 9:662-681.
Taylor DP, Netscher C (1974). An improved technique for preparing perineal patterns of Meloidogyne spp. Nematologica 20:268-269
Trudgill DL (1991). Resistance to and tolerance of plant parasitic nematodes in plants. Annual Rev. Phytopathol. 29:167-193.
Williamson VM, Kumar A (2006). Nematode resistance in plants: the battle underground. Trends Genet. 22:396-403.