RESEARCH/INVESTIGACIÓN

DISTRIBUTION OF *MELOIDOGYNE CHITWOODI* mtDNA HAPLOTYPES IN THE MAJOR POTATO PRODUCTION AREAS OF WASHINGTON STATE

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ABSTRACT

Humphreys-Pereira, D. A., T. L. Peever, and A. A. Elling. 2022. Distribution of *Meloidogyne chitwoodi* mtDNA haplotypes in the major potato production areas of Washington State. Nematropica 52:64-71.

Washington is ranked second in potato production in the United States based on total yield. One of the major threats to the potato industry in the Pacific Northwest (Oregon, Washington, and Idaho) is root-knot nematodes, particularly *Meloidogyne chitwoodi*. The distribution and genetic diversity of *M. chitwoodi* in the region are unknown. Eighteen tuber samples (up to ten tubers per sample) showing typical galls induced by root-knot nematodes were collected from the top six potato-producing counties in Washington. Species identification was performed using PCR with species-specific primers. Eighteen *Meloidogyne* samples were identified as *M. chitwoodi* whereas concomitant *Meloidogyne* species (*M. chitwoodi* and *M. hapla*) were found in two samples. Intraspecific variability of *M. chitwoodi* was studied by sequencing the mitochondrial (mt) locus cytochrome b (*cytb*). Three *cytb* haplotypes were identified based on four segregating sites detected from an alignment of 540 sequences [each corresponding to one second-stage juvenile (J2)]. Haplotype 1 represented 71% of *M. chitwoodi* J2 (382 sequences), followed by haplotype 2 with 19% (100 sequences), and haplotype 3 with 11% (58 sequences). All three haplotypes were identified in a sample from Quincy, Grant County, the same location from which *M. chitwoodi* was first described in the 1980s.

Key words: Columbia root-knot nematode, *cytb* gene, diagnosis, distribution, *M. hapla*

RESUMEN

Humphreys-Pereira, D.A., T. L. Peever, y A.A. Elling. 2022. Distribución de haplotipos ADNmt de *Meloidogyne chitwoodi* en las áreas de mayor producción de papa en el Estado de Washington. Nematropica 52:64-71.

Washington se encuentra en segunda posición en producción de papa en los Estados Unidos basados en rendimiento total. Una de las mayores amenazas para la industria de la papa en el Pacífico Noroeste (Oregón, Washington and Idaho) es el nematodo agallador, particularmente *Meloidogyne chitwoodi*. La distribución y diversidad genética de *M. chitwoodi* en el estado se desconocen. Dieciocho muestras de tubérculos de papa (hasta diez tubérculos por muestra) mostrando agallas típicas inducidas por nematodos agalladores se recolectaron en los seis condados de mayor producción de papa en Washington. La identificación a nivel de especie se realizó utilizando PCR con imprimadores específicos de especie.

Dieciocho muestras de Meloidogyne se identificaron como *M. chitwoodi* mientras que especies de *Meloidogyne* concomitantes (*M. chitwoodi* and *M. hapla*) se encontraron en dos muestras. La variabilidad intraespecífica de *M. chitwoodi* se estudió con la secuenciación del locus mitocondrial (mt) citocromo b (*cytb*). Tres haplotipos *cytb* se identificaron basados en cuatro sitios segregantes identificados de un alineamiento de 540 secuencias [cada una correspondió a un juvenil en segundo estado (J2)]. Haplotipo 1 representó 71% de J2 de *M. chitwoodi* (382 secuencias), seguido de haplotipo 2 con el 19% (100 secuencias) y haplotipo 3 con el 11% (58 secuencias). Los tres haplotipos se identificaron en una muestra de Quincy, del condado de Grant, la misma localidad de donde se describió por primera vez *M. chitwoodi* en los 1980s.

Palabras clave: Nematodo agallador de Columbia, gen *cytb*, diagnóstico, distribución, *M. hapla*

INTRODUCTION

Washington ranked second in potato production in the United States in 2019 with a total yield of 103 million cwt or about one fourth of the nationwide total yield (USDA-NASS, 2020). Potatoes ranked fourth among the top ten commodities in Washington with a farmgate value of $687 million, just behind apples, milk, and wheat (USDA-NASS, 2018). The major potato-producing counties (represented by hectares harvested in 2017) in Washington are Grant Co. (19,041 ha), followed by Benton Co. (17,015 ha), Franklin Co. (12,133 ha), Adams Co. (8,814 ha), Walla Walla Co. (4,137 ha), and Skagit Co. (4,004 ha) (USDA-NASS, 2019a).

One of the major threats to potato production in many potato-producing regions worldwide, including the Pacific Northwest (PNW) of the United States, is the Columbia root-knot nematode (CRKN), *Meloidogyne chitwoodi* (Elling, 2013). The wide host range of *M. chitwoodi*, which includes monocotyledons and dicotyledons (O'Bannon *et al.*, 1982) and the presence of races and pathotypes able to reproduce on potatoes carrying the resistance gene $R_{Mc1(blb)}$ (van der Beek *et al.*, 1999; van der Beek and Poleij, 2008; Brown *et al.*, 2009;) make crop or cultivar rotation as a control tactic for this nematode largely ineffective.

Current information about the distribution and genetic diversity of *M. chitwoodi* is largely lacking but is important to provide insights into the geographical origin of *M. chitwoodi* and offer critical information for potato-breeding programs. The area of a species origin is commonly associated with a high genetic variability, which provides useful knowledge for these programs. The aims of the present study were to: (i) determine the distribution of *Meloidogyne* species in the major potato-producing areas in Washington and (ii) assess the intraspecific variability of *M. chitwoodi* based on the cytochrome b (*cytb*) mitochondrial marker.

MATERIALS AND METHODS

Nematode sample collection

Eighteen *Meloidogyne* samples from the top six potato-producing counties (based on hectares harvested) in Washington were collected between 2012 and 2015 (Table 1). Two more *Meloidogyne* samples from Idaho and Oregon were included in this study. Twenty tuber samples per location were collected from potato-packing storage facilities or received from commercial growers. A subsample of 5-10 tubers showing typical gall symptoms on the surface were selected from each of these 20 tuber samples. Second-stage juveniles (J2) were extracted from these subsamples following procedures of Humphreys-Pereira *et al.* (2014a) and Caveness and Jensen (1955). Briefly, tubers were peeled, and the peel was chopped into 1-2 cm pieces and mixed in a bag. Ten grams of the peel was processed with the flotation-centrifugation method. The peel was ground with a blender and the suspension poured through sieves no. 100 (149-µm pore size) and no. 500 (25-µm pore size). The eggs and J2 retained on the 500-mesh sieve were transferred to 50-ml tubes and centrifuged for 3 min at 3,000 rpm. The supernatant was discarded, and the tubes was filled with a sucrose solution (471 g sucrose/1 L water). Tubes were centrifuged one more time for 3 min at 3,000 rpm and the supernatant poured over a 500-mesh sieve. Tap water was added to the sieve to wash the sucrose from the nematodes, which were transferred to a beaker for further analysis.

Table 1. Host and origin of extracted *Meloidogyne chitwoodi* samples and mitochondrial *cytb* haplotypes.

Sample code	Host	Sample origin	*cytb* haplotypes[z]
S1	Potato cv. Ranger Russet	Franklin County, WA	20 J2 = H1
S2	Potato cv. Alpine	Paterson, Benton Co., WA	20 J2 = H1
S3	Potato cv. Ranger Russet	Pasco, Franklin Co., WA	20 J2 = H1
S4	Potato cv. Alturas	Pasco, Franklin Co., WA	20 J2 = H1
S5	Potato cv. Ranger Russet	Prosser, Benton Co., WA	20 J2 = H1
S6	Potato cv. Russet Norkotah	Franklin Co., WA	19 J2 = H1, 1 J2 = H2
S7	Potato	Grant Co., WA	20 J2 = H1
S8	Potato	Hermiston, Umatilla Co., OR	20 J2 = H2
S9	Potato cv. Russet Burbank	Quincy, Grant Co., WA	13 J2 = H1, 8 J2 = H2, 19 J2 = H3
S10	Potato cv. Russet Burbank	Cassia Co., ID	1 J2 = H1, 39 J2 = H3
S11	Potato cv. Chieftain	Skagit Co., WA	20 J2 = H1
S12	Potato	Othello, Adams Co., WA	20 J2 = H1
S13	Potato cv. Yukon	Pasco, Franklin Co., WA	20 J2 = H1
S14	Potato cv. Umatilla	George, Grant Co., WA	6 J2 = H1, 14 J2 = H2
S15	Potato cv. Russet Burbank	Burbank, Walla Walla Co., WA	20 J2 = H1
S16	Potato cv. Russet Burbank	Connell, Franklin Co., WA	20 J2 = H1
S17	Potato cv. Yukon	Pasco, Franklin Co., WA	20 J2 = H1
S18	Potato cv. Alturas	Pasco, Franklin Co., WA	20 J2 = H1
S19	Potato cv. Chieftain	Burlington, Skagit Co., WA	20 J2 = H1
S20	Potato cv. Satina	Burlington, Skagit Co., WA	20 J2 = H1
NM	Potato	San Juan Co., NM	20 J2 = H1
WAMC1	Pure culture on tomato cv. Rutgers	Quincy, Grant Co., WA	20 J2 = H2
WAMCRoza	Pure culture on tomato cv. Rutgers	Prosser, Benton Co., WA	20 J2 = H2
WAMC27	Pure culture on tomato cv. Rutgers	Washington	3 J2 = H1, 17 J2 = H2
CAMC2	Pure culture on tomato cv. Rutgers	Tulelake, Siskiyou Co., CA	20 J2 = H1

[z]H1, H2, and H3 are the different *cytb* haplotypes found in the *M. chitwoodi* samples. A total of 540 individual J2 were analyzed.

Positive controls included *M. chitwoodi* races 1 (WAMC1) and 2 (WAMC27), pathotypes WAMCRoza (RO) and CAMC2 (CA) (Humphreys-Pereira and Elling, 2013), an isolate from San Juan Co., New Mexico (NM), and a positive control of *M. hapla* (MH) from Prosser, WA. Including the controls, a total of 25 *Meloidogyne* populations were analyzed in the study.

DNA extraction

Crude DNA of single J2 was extracted according to Williamson *et al.* (1997) with some modifications (Sandoval-Ruiz *et al.*, 2020). Briefly, 24 single J2 were randomly picked from tuber extracts and transferred into separate PCR tubes containing 10 µl DreamTaq PCR reaction buffer (Thermo Fisher Scientific, Pittsburgh, PA), 10 µl water and 1.5 µl proteinase K (20 mg/ml; New England BioLabs, Ipswich, MA). Tubes were incubated at 58ºC for 16 h and then at 95ºC for 20 min. Bulk nematode DNA was extracted from each of the 25 *Meloidogyne* samples using the QIAamp DNA Micro Kit (Qiagen, Stanford, CA). For bulk extractions, 100 J2 from each *Meloidogyne* sample were combined in a single microcentrifuge tube containing glass beads (Sigma-Aldrich, St. Louis, MO), 280 µl ATL buffer and 20 µl proteinase K (20 mg/ml; Qiagen, Stanford, CA). The tubes were transferred to a bead beater for maceration, and DNA was extracted following the manufacturer's protocol.

Molecular characterization and sequence analysis

Primers JMV/JMV2/JMVhapla (Wishart *et al.*, 2002) were used for the identification of

Meloidogyne to the species level. PCR amplification and conditions were followed according to Humphreys-Pereira *et al.* (2014b) with both types of DNA samples, crude DNA from single J2 and bulk DNA samples. Only crude DNA from single J2 was used for the the *cytb* gene study. Primers Mch*cytb*F (5′-TGGTGTGGATTTTGTTTTAAATTATT-3′) /MCh*cytb*R (5′-GATAACTCAACAAATGGATAAATTGG-3′) for the cytochrome b (*cytb*) gene were designed using the mitochondrial (mt) genome sequence of *M. chitwoodi* (Humphreys-Pereira and Elling, 2014) and optimized with a gradient PCR. The reactions included 0.4 µM of each primer, 1× Phusion HF Buffer (New England Biolabs), 200 µM dNTP mix (New England Biolabs), 0.5 U Phusion DNA polymerase (New England Biolabs) and 2 µl of crude DNA from a single *Meloidogyne* J2, in a final volume of 25 µl. PCR reactions were carried out in Mastercycler pro thermal cycler (Eppendorf, Hamburg, Germany) following the amplification conditions of initial denaturation at 98°C for 30 sec, followed by 35 cycles of denaturation at 98°C for 5 sec, annealing at 60°C for 20 sec, extension at 72°C for 30 sec with a final extension step at 72°C for 2 min. PCR products from at least 20 *Meloidogyne* J2 were sequenced bidirectionally at Elim Biopharmaceuticals (Hayward, CA). Sequences were analyzed using Bioedit version 7.0.9 (Hall, 1999) and aligned using the ClustalW algorithm (Thompson *et al.*, 1994). Pairwise distances (%) were calculated using MEGA7 (Kumar *et al.*, 2016). A total of 540 sequences with a length of 891 bp was obtained from the 25 *Meloidogyne* samples.

RESULTS

Meloidogyne *species identification*

PCR amplification using the specific primers JMV1/JMV2/JMVhapla resulted in a single 540 bp product amplified from 23 of 25 bulk DNA samples, confirming that they were *M. chitwoodi* (Fig. 1A). A two-band pattern was observed in two bulk DNA samples (S15 and S16), with product sizes of 540 bp and 440 bp, typical for *M. chitwoodi* and *M. hapla*, respectively. These primers were also tested on single *Meloidogyne* J2 from all samples and confirmed the presence of concomitant *Meloidogyne* species in two composite potato tuber samples. *Meloidogyne chitwoodi* was found in the Washington counties Franklin, Benton, Grant, Adams, Walla Walla and Skagit, as well as in Umatilla Co. in Oregon and Cassia Co. in Idaho (Table 1). *Meloidogyne hapla* was identified in potato tubers from Walla Walla, and Franklin counties in Washington. All white, red, and yellow potato cultivars sampled in this study (Table 1), showed typical gall symptoms on the tuber surface and the presence of *M. chitwoodi*.

Genetic variation of Meloidogyne chitwoodi *populations in the* cytb *gene*

PCR amplification using the primer set Mch*cytb*F/Mch*cytb*R resulted in an amplicon of ~900 bp. The *cytb* gene marker was sequenced from at least 20 *M. chitwoodi* J2 per sample (except S9 and S10 with 40 *M. chitwoodi* J2, each), for a total of 540 *M. chitwoodi* J2 individuals from 25 samples (including the positive controls from Washington, California and New Mexico). The alignment of all sequences revealed three mt haplotypes for *M. chitwoodi* based on four substitutions, and were designated as H1, H2, and H3 (GenBank accession numbers MT882026-MT882028) (Table 2). One of the four substitutions was synonymous and the other three were non-synonymous. Sequence divergence levels among all sequences ranged from 0 to 0.45% (0 to 4 bp). Haplotype H1 represented 71% of individuals (382 *M. chitwoodi* J2), followed by haplotype H2 with 19% (100 *M. chitwoodi* J2), and haplotype H3 with 11% (58 *M. chitwoodi* J2). All three *M. chitwoodi* haplotypes were present in the sample S9 from Quincy, Grant Co., WA (Table 1, Fig. 1B). Four samples had two haplotypes (S6, S10, S14, and the control WAMC27) and the remaining samples had a unique haplotype. Only two samples had haplotype H3, one from Quincy, WA and from Cassia Co., ID (samples S9 and S10).

DISCUSSION

Most of the potato samples analyzed in this study originated from the major potato-producing counties in Washington, including Grant, Benton, Franklin, Adams, Walla Walla, and Skagit (USDA-NASS, 2019a). PCR with species-specific primers followed by sequencing of the *cytb* gene allowed for the identification of *M. chitwoodi* in all potato tuber samples, which represented some of the most

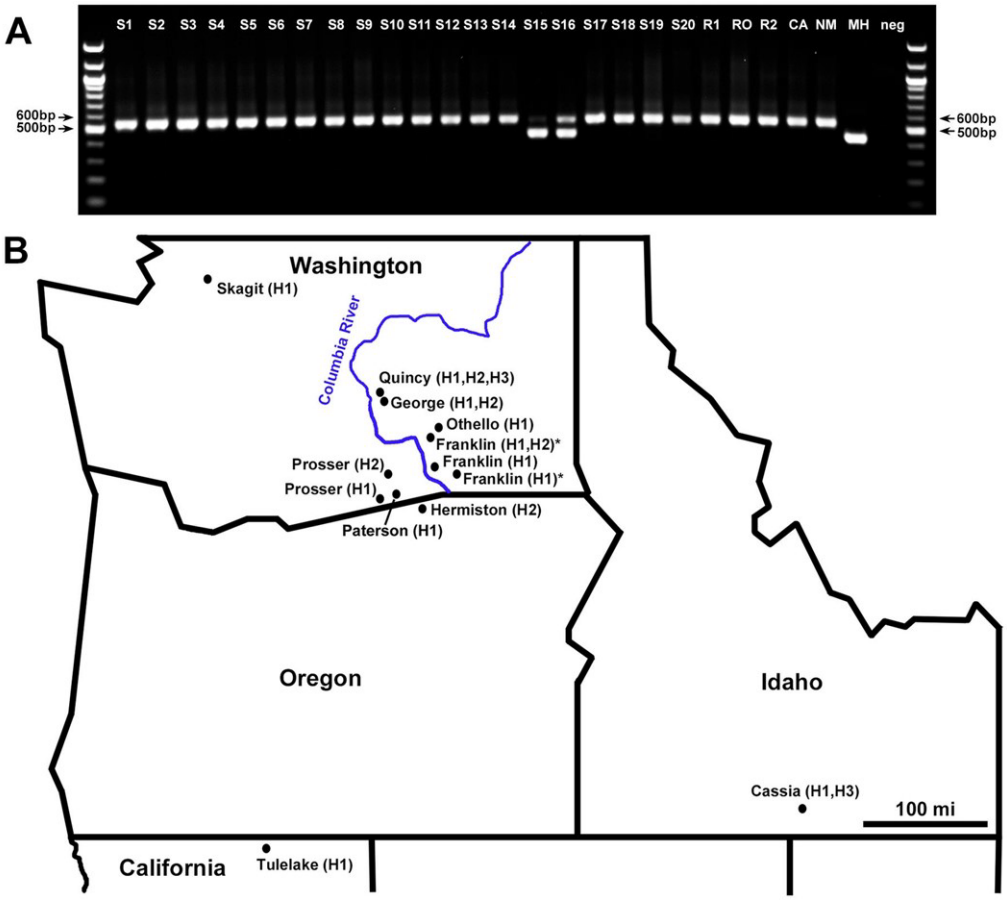

Figure. 1. A: Molecular identification of *Meloidogyne chitwoodi* and *M. hapla* using the species-specific primers JMV1/JMV2/JMVhapla in bulk DNA samples from 25 *Meloidogyne* samples. S1 to S20 = *Meloidogyne* samples extracted from potato tubers. R1 (WAMC1), RO (WAMCRoza), R2 (WAMC27), CA (CAMC2), NM (New Mexico) = *Meloidogyne chitwoodi* positive controls. MH = *M. hapla* positive control. neg = negative control. First and last lanes represent 100-bp marker ladder (New England BioLabs). B: Distribution of the mitochondrial haplotypes (H1, H2 and H3) based on the *cytb* gene from 25 *Meloidogyne chitwoodi* samples. Black dots indicate the origin of tuber samples, with several samples from some areas. Asterisks represent samples with a mix of concomitant species, *M. chitwoodi* and *M. hapla*.

Table 2. Substitutions in the alignment of the three *Meloidogyne chitwoodi* mitochondrial *cytb* haplotypes.

Nucleotide position		Haplotype			
Within complete gene	Within amplified product	H1	H2	H3	Reference mt genome[z]
284	245	A	A	A	A
511	472	A	A	T	A
543	504	T	T	A	T
909	870	A	A	A	A

[z]Humphreys-Pereira and Elling (2014).

widely planted cultivars in Washington, e.g. Russet Burbank (29.6% of planted acres), Umatilla (14.8%), Ranger and Russet Norkotah (both 8.1%), Alturas (3.7%), and Chieftain (2.6%) (USDA-NASS, 2019b). Typical gall symptoms on the tuber surface and the presence of *M. chitwoodi* might

suggest the lack of resistance in these cultivars. In two samples, *M. hapla* and *M. chitwoodi* were identified concomitantly. Similarly, a survey in five northwestern states of the United States dated 1980-1981 showed that the predominant *Meloidogyne* species infecting potatoes was *M. chitwoodi* with 83% of the samples (total of 187 samples), whereas *M. hapla* was found in 11%, and both species were found concomitantly in 6% of the samples (Nyczepir *et al.*, 1982). Recently, Zasada *et al.* (2019) based on a dataset of 9,383 samples across the Pacific Northwest (Oregon, Idaho, and Washington) from 2012 to 2016 showed that *M. chitwoodi* was the most common *Meloidogyne* species in the region with a frequency of 65%, followed by *M. hapla* with a frequency of 25%, and a mix of *M. chitwoodi* and *M. hapla* in 12% of the samples.

The less frequent *cytb* haplotype, H3, was found only in Quincy, WA and in Cassia Co., ID. Importantly, all three *cytb* haplotypes were found in Quincy, WA, the same location where *M. chitwoodi* was first described in the 1980s (Golden *et al.*, 1980). Taken together, the results of this study not only document the genetic diversity of *M. chitwoodi* in the most important potato-producing counties in Washington, but also add important information for future studies aimed at identifying the geographic and evolutionary origin of *M. chitwoodi*. Analyses of mitochondrial haplotypes within the genus *Meloidogyne*, such as with the nad5 gene, have provided a useful barcode for the identification of tropical root-knot nematodes species (*M. incognita* group, MIG) and has shed light on their evolution (Janssen *et al.* 2016). In addition, the *cytb* DNA marker differentiated *M. ethiopica*, *M. floridensis* and *Meloidogyne* sp. 2 and delineated three haplotypes within *M. arenaria*, similar to our findings in the temperate species, *M. chitwoodi*. In other nematode genera, the *cytb* fragment was also used to investigate the origin of western European populations of *Globodera pallida* (Plantard *et al.*, 2008). More *cytb* haplotypes were found in Peru than in Western Europe, and it was inferred from a phylogenetic analysis that the western European populations of *G. pallida* could have originated in the south of Peru. Furthermore, Madani *et al.* (2010) concluded based on *cytb* sequences that Canadian *G. pallida* populations were introduced from Europe. Similarly, our findings and the identification of three *M. chitwoodi cytb* haplotypes in Washington set the stage for future studies focused on the phylogenetics and possible routes of introduction of this important quarantine pathogen.

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