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CYTOGENETIC STATUS OF ROOT-KNOT NEMATODES IN JORDAN

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ABSTRACT

Three species of the root-knot nematodes (RKNs), *Meloidogyne javanica*, *M. incognita* (races 1 and 2) and *M. arenaria* (race 2) were found to occur in Jordan. The species and their races were identified using morphological characters, the North Carolina differential host test, and SCAR based PCR assay. Cytogenetical preparations for the study of gametogenesis and chromosome numbers were made using propionic-orcein dye for chromosome staining and Giemsa stain for result comparison. Results indicated that the populations of *M. incognita* race 1, *M. incognita* race 2, and *M. javanica* were considered hypotriploid with an average of 45.2, 46.1 and 46.7 chromosomes, respectively. The *M. arenaria* populations, however, were considered triploid with an average of 52.2 chromosomes. The chromosomal numbers suggested that the mode of reproduction for the three species was mitotic parthenogenesis.

Keywords: *Meloidogyne javanica*, *M. incognita*, *M. arenaria*, parthenogenesis.

1. INTRODUCTION

The genus *Meloidogyne* comprises a widely distributed group of plant-parasitic nematodes that attack a wide range of crop species.

In Jordan, Abu-Gharbieh (1982) identified two Meloidogyne species; *M. javanica* (Teurb) Chitwood and *M. incognita* (Kofoid & White) Chitwood from soil and plant samples collected from the major irrigated areas of Jordan. The average annual losses of irrigated vegetable crops cultivated in the Jordan Valley due to RKNs are estimated by nearly 15 % (Abu-Gharbieh, 1994).

Meloidogyne species can reproduce by apomixis, facultative meiotic parthenogenesis or obligate mitotic parthenogenesis (Abad *et al*., 2003). The cytogenetic status of the genus Meloidogyne is complex but important in understanding the overall biology of these nematodes (Moens and Pery, 2013). Root-knot nematodes have undergone extensive cytogenetic diversification, probably unparalleled by that of any other animal group (Triantaphyllou, 1985). Characteristic features are the establishment of meiotic and mitotic parthenogenesis in association with various degrees of polyploidy and aneuploidy. Obligatory cross-fertilization also occurs in some diploid and polyploid forms, whereas facultative meiotic or obligatory mitotic parthenogenesis prevails in most polyploid and aneuploid forms (Eisenback and Triantaphyllou, 1991). This extensive cytogenetic diversification must have played an important role in the overall biological and

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genetic evolution of these nematodes (Triantaphyllou, 1990). The accurate identification of RKN species (*Meloidogyne* spp.) is essential for implementing management strategies. Methods based on the morphology of adults, isozymes phenotypes and DNA analysis including cytogenetics can be used for the diagnosis of RKN (Cunha *et al.* 2018).

The objective of this study was to assess the cytogenetical composition of *Meloidogyne* species and races prevailing in the irrigated areas of Jordan.

2. MATERIALS AND METHODS

2.1 Collection of samples

Eighty-three soil and galled root samples were collected from infected vegetable crops and fruit trees. The survey covered most of the irrigated agricultural areas with different climatic conditions: Southern Ghors, Jordan Valley, the elevated uplands, and the eastern desert plains.

2.2 Population identification

Populations derived from a single egg-mass were obtained by inoculating individual egg-masses on the susceptible tomato cv. GS 12. The identification of the 83 *Meloidogyne* (its taxonomic status is shown in Figure 1) populations was based on the perineal pattern morphology (Barker *et al*., 1985) and North Carolina differential host test (Hartman and Sasser, 1985), and DNA fingerprinting assay using the sequence characterized amplified regions-polymerase chain reaction (SCAR-PCR) developed by Zijlstra *et al.* (2000). Data on these identification procedures are not presented herein but in previous studies (Abu-Gharbieh *et al.* 2005; Karajeh *et al.* 2005 and 2006).

2.3 Cytogenetic studies

Cytogenetical preparations for the study of gametogenesis and chromosome counting were made for all 83 populations of the RKNs from Jordan according to Triantaphyllou (1985). Propionic-orcein dye for chromosome staining and Giemsa stain for result comparison were used.

2.3.1 Preparation of chromosomal stains

Propionic-orcein stain was prepared by addition of 2.2 gm synthetic orcein stain (Fluka, USA) to 100 ml of propionic acid and boiled gently for 30 minutes. The solution was cooled and then diluted by addition of 100 ml of distilled water. The solution was passed through a fine filter paper for removal of any undissolved stain particles (Triantaphyllou, 1985). Giemsa stain stock was prepared by dissolving 3.8 grams Giemsa stain powder in 25 ml of glycerin, gently heated at 60° C with stirring for about 2 hours, and allowed to cool to room temperature before adding 75 ml methanol. For preparation of the working solution, the stock solution was diluted before use to 10^{-1} with water (Gustashaw, 1991).

2.3.2 Preparation of smears, hydrolysis and fixation

For smear preparation, four gravid females, extracted from galled roots, were drawn using a fine pipette, and transferred with a small drop of water on a clean slide. The females were then smeared in parallel strips and spaced 2 mm from each other. Smears were allowed to dry for 3-5

minutes before proceeding to the hydrolysis step. Slight hydrolysis was carried at room temperature before fixation. A solution of 1N hydrochloric acid (HCl) was used for smear hydrolysis before the nematode material had dried excessively. The slide was left in the HCl solution for 5-10 minutes at room temperature. The slide was immersed in a glass jar with freshly prepared fixative consisting of three parts of absolute ethanol and one part of glacial acetic acid for 20-30 minutes.

2.3.3 Staining and mounting

The slide was removed from the fixative and wiped dry with tissue paper leaving wet only the smears and a small area surrounding them. Two drops of propionic orcein stain were applied on the smears then the stain was covered with a deep-well (cavity) slide. The smears were allowed to stain for 20-40 minutes followed by mounting.

After removal of the cavity slide, the slide was held with smears vertically on a piece of tissue paper to drain the excess propionic orcein stain. To remove the rest of the stain, the slide was immersed for 3-5 seconds in a glass jar containing 45% propionic acid. The slide was then removed from the 45% propionic acid and placed on absorbent paper on the working table. A 22-mm-square cover slip was dipped momentarily in the 45% propionic acid to wet it and then applied on the wet smears. The slide was allowed to dry slowly for 5 minutes and then sealed with fingernail paint. Alternatively, some slides were stained with Giemsa stain by adding 1-3 drops of Giemsa stain on the smears for 5 minutes. The slides were then rinsed for a few seconds under tap water. The slide was allowed to dry for 10 minutes then left uncovered.

2.3.4 Microscopic examination of prepared slides

Stained slides with either orcein or Giemsa were carefully examined microscopically within 3-5 days. Young oocytes were viewed through slide stereoscopic microscope and the number of chromosomes per oocyte was counted. An average of 10 chromosome number reading was made at the pro-metaphase stage. A tabulated summary of cytogenetic information (Triantaphyllou, 1985) related to RKNs was used as a reference guide to determine the mode of reproduction.

Means, standard deviations, ranges, and coefficients of variation for cytogenetic data of the populations were calculated. Mean separation was determined using Duncan's multiple range test (DMRT) at the 0.05 probability level (Steel and Torrie, 1980).

3. RESULTS

Seventy of the total 83 populations collected from Jordan (84%) were identified as *M. javanica*, 5 (6%) as *M. incognita* race 1, 3 (4%) as *M. incognita* race 2 and 5 (6%) as *M. arenaria* race 2.

Both orcein and Giemsa stains were found useful in this nematode cytogenetic study. However, in orcein stain preparations the chromosomes appeared in larger than those in Giemsa stain preparations that facilitated easy chromosome counting.

Cytologenetical observations to determine the chromosome number of each population were made primarily on oocytes located in the anterior part of the uterus, adjacent to the spermatheca. Such oocytes were expected to be at late prophase or metaphase of the first maturation. All populations showed similar configuration that consisted of univalent chromosomes suggesting

that no pairing of homologous chromosomes (no sexual reproduction) had occurred earlier and that all populations reproduced by mitotic parthenogenesis.

Meloidogyne incognita populations exhibited the typical distribution of chromosomes for this species; which was characterized by an unusual clustering of the chromosomes. The populations of *M. incognita* race 1 (P15-P17, P74 and P75) had chromosome number ranging from 41.1-48.0 with an average of 45.26 chromosomes and were considered hypotriploid. The *M. incognita* race 2 (P31, P32 and P82) populations had chromosome number ranging from 45.2-46.6 with an average of 46.1 chromosomes and were also considered hypotriploid. The *M. javanica* populations showed chromosome number ranging from 43.0-48.7 and are considered hypotriploid with an average of 46.7 chromosomes. However, *M. arenaria* populations (P26-30) had chromosome number ranging from 50.8-54.3 and were considered triploid with an average of 52.26 chromosomes (Table 1 and 2). All of these chromosomal numbers also suggested that the mode of reproduction was mitotic parthenogenesis. The chromosomal number for *M. arenaria* race 2 oocytes, was significantly higher than those of the other species and races (Table 1 and 2). The coefficients of variation were less than 10% suggesting possible usefulness of this trait (chromosomal number) in differentiating *M. arenaria* race 2 from the other populations in Jordan.

4. DISCUSSION

Meloidogyne species exhibit extensive cytogenetic diversity, with many parthenogenetic forms, a large proportion of which are polyploid, obligate mitotic parthenogens demonstrating a broad geographical distribution and host range diversity (Siddiqi, 2000). To date, over 80 species of *Meloidogyne* have been described, three of which are extremely polyphagous parthenogenetic species: *M. incognita*, *M. javanica*, and *M. arenaria* that are distributed worldwide and account for the majority of crop losses due to RKNs (Xu *et al.*, 2001). All of the three major *Meloidogyne* species were found in Jordan. *Meloidogyne arenaria* is a new record reported from Jordan for the first time in these studies.

All *Meloidogyne* populations studied were found to reproduce obligatorily by mitotic parthenogenesis. No meiotic facultatively amphimictic populations were found. *Meloidogyne arenaria* constituted the only triploid form that is the most representative cytogenetic form of this species worldwide. Meanwhile, all populations of *M. javanica* and *M. incognita* were hypotriploid, which is the only cytogenetic form known for these two species worldwide. This is the first reported study of cytogenetics on plant parasitic nematodes in Jordan. Therefore, *M. arenaria* can be differentiated from the other two *Meloidogyne* species in Jordan based on their higher number of chromosomes. Freire *et al*. (2002) used cytogenetics to differentiate *Meloidogyne* species from Brazile. Triantaphyllou (1988) used the cytogenetic features as taxonomic characters for species identification and in the interpretation of phyletic relationships of RKNs.

5. CONCLUSION

In this study, the use of cytogenetic data complimented other data including nematode morphology and DNA fingerprinting in presenting a sound assessment for the taxonomic status

of *Meloidogyne* species and races which is expected to provide an essential background for the management of RKNs in Jordan.

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Figure 1: Taxonomic status of root-knot nematodes:

Kingdom: Animalia

Phylum: Nematoda

Class: Secernentea

Order: Tylenchida

Suborder: Tylenchina

Superfamily: Heteroderoidea

Family: Meloidogynidae

Genus: *Meloidogyne*

Table 1: Summary of the cytogenetic characteristics of *Meloidogyne* species and races in Jordan.

Nematode species	***M. javanica* (70)** [1]	***M. arenaria* race 2 (5)**	***M. incognita* race 1 (5)**	***M. incognita* race 2 (3)**
Number of chromosomes	46.72 [2a]b ± 1.09 [2b] (43.0-48.70) [2c] 2.3 [2d]	52.26a ± 1.45 (50.8-54.3) 2.8	45.26b ± 2.64 (41.10-48.00) 5.8	46.1b ± 0.78 (45.20-46.60) 1.7
Mode of Reproduction	hypotriploid	triploid	hypotriploid	hypotriploid

(1) Number of populations.
(2a) Average of around 10 females; (2b) Standard deviation; (2c) Range (minimum-maximum); (2d) Coefficient of variation.
(3) Means followed by the same letter within columns are not significantly different according to DMRT ($P< 0.05$).