MOLECULAR IDENTIFICATION OF THE ROOT KNOT NEMATODES *Meloidogyne* spp. IN THE AUTONOMOUS PROVINCE OF VOJVODINA IN SERBIA

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ABSTRACT

The root knot nematodes (RKN) of the genus *Meloidogyne* are among the most destructive plant-parasitic polyphagous organisms. They are distributed worldwide and infest the root system, causing abnormal swellings, called galls, which lead to a reduction in crop yield and quality of plant products. Molecular identification of RKN was carried out in the frame of the official *Meloidogyne* survey as part of the activities of the Annual Plant Health Programme, which is financially supported by the Directorate for Plant Health of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. The molecular equipment was financially supported by Provincial Secretariat for Agriculture, Water Management and Forestry in the frame of project "Development and implementation of molecular methods of identification in Plant Nematology". Soil and plant material samples were collected in 2023 in 7 districts of the province Vojvodina in Serbia in greenhouses and open fields. A total of 45 samples came from vegetables and ornamental plants and 26.7% (12 samples) were infested with *Meloidogyne* spp. Twelve samples (populations) were identified using a combination of morphological approach based on perineal patterns and molecular approach with PCR and species-specific primers. Three *Meloidogyne* species were determined on 12 locations in 5 districts: *Meloidogyne incognita* (9 samples), *M. hapla* (2 samples) and *M. arenaria* (1 sample). The results showed that *M. incognita* is the predominant RKN species in the province of Vojvodina in Serbia. The correct identification of RKN species is the first step towards efficient pest control that ensures healthy plants and good yields. Therefore, a national survey of RKN in Serbia is also planned for the future.

Keywords: distribution, *Meloidogyne* spp., molecular identification, morphological identification, Vojvodina, Serbia**.**

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1 INTRODUCTION

Root-knot nematodes (RKN), the genus *Meloidogyne* are among the most damaging groups of plant parasitic nematodes. These obligate polyphagous organisms cause swellings on root system called galls. More than 90 species from this genus were identified worldwide (Hunt and Handoo, 2009; Moens *et al*., 2009) and four *Meloidogyne s*pecies (*M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*) are the most important crop pests in agriculture (Karssen, 2002). Six RKN species (*M. chitwoodi, M. fallax, M. enterolobii, M. mali, M. graminicola, M. luci*) were added to the European list of quarantine nematodes (EPPO, 2003). The Vojvodina Province, northern part of Serbia had prevalent vegetable production in field (95%) and 5% of production in glasshouses (Červenski *et al*., 2015). Five *Meloidogyne* species (*M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla, M. naasi*) were detected on vegetables, ornamental plants and grasses in Vojvodina Province until 1990's (Grujičić, 1959; Grujičić, 1967; Grujičić and Borić, 1971; Jovičić and Grujičić, 1986). During 1990's there were not new reports on RKN in this region. In the period of 2014-2018, highly damages on potato crops (*Solanum tuberosum* L.) caused by *M. incognita* and *M. arenaria* were observed on the Serbian-Hungary border (Bačić *et. al*., 2016; Bačić *et. al*., 2022a). Bačić *et al.* (2022b; 2023) reported *M. arenaria* for the first time on calla (*Calla palustris* L.) and *M. luci* on tomato (*Solanum lycopercisum* L.) in Vojvodina Province. The purpose of this study was to identify *Meloidogyne* species in the province of Vojvodina in Serbia in the frame of the official RKN survey in 2023 using morphological and molecular methods in order to update the knowledge about the occurrence and distribution of *Meloidogyne* species in this region. Accurate RNK identification based on morphological and molecular methods is important for effective management and control of these pests which has been implemented in Serbia.

2 MATERIALS AND METHODS

Plant material samples were collected in 2023 in 7 districts of Vojvodina Province in Serbia (North Bačka, West Bačka, South Bačka, Central Banat, North Banat, South Banat and Srem district) in the frame of official *Meloidogyne* survey of Program of Measures in Plant Health by the Serbian Plant Protection Directorate of Ministry of Agriculture, Forestry and Water Management (MAFWM). In total, 45 samples of symptomatic and asymptomatic plant material originated from greenhouses and fields were analysed. The females and egg mass were removed from the roots using needles under stereomicroscope (Nikon SMZ 800N) with a 40x magnification. Morphological identification was performed on females by perineal patterns analysis under microscope (Leica DM 1000 LED) at 400x magnification. Perineal patterns were prepared following the procedures suggested by Hartman and Sasser (1985). Species level identification was made according to Karssen (2002). Molecular identification was carried out according to Wishart *et al*. (2002) and Adam *et al*. (2007). Samples of egg mass were pooled in 150 µl of sterile water, and isolation of total DNA was performed with the Qiagen Blood and Tissue Kit (Qiagen, Crawley, West Sussex, UK), according to the manufacturer's instructions. PCR amplification reactions were carried out in 25 µl reactions comprised of 12,5 µl Taq Ready Master mix (Fast Gene), 0.5 µl of each primer

(10 µmol),1 µl DNA and 10,5 µl ddH2O. The amplification was carried out using the following program: one cycle of initial denaturation at 95 °C for 3 minutes; 45 cycles of denaturation at 94 °C for 30 seconds, annealing at: 50 °C (194/195); 50 °C (JMV primers); 61 °C (Far/Rar); 62 °C (MI-F/MI-R); 64 °C (Fjav/Rjav), elongation at 72 °C for 90 seconds (194/195; JMV primers) and 60 seconds for all other primers; followed by final elongation at 72 °C for 7 minutes. Depending on the primer that was used, the following bands were expected when amplified PCR products were shown on a 1% agarose gel. The genus *Meloidogyne* was first divided into three groups with the expected bands based on PCR primers 194/195: *M. hapla* (700 base pairs), *M. incognita*, *M. arenaria*, *M. javanica* (720 bp), *M. mayaguensis* (780 bp), and *M. chitwoodi* and *M. fallax* (between 1600 and 1700 bp). Primers MI-F and MI-R specific for the species *M. incognita* (999 bp), Fjav and Rjav specific for the species *M. javanica* (720 bp), Far and Rar specific for the species *M. arenaria* (420 bp), and primers JMV1, JMV2, JMV hapla and JMV tropical specific for the species *M. chitwoodi* (540 bp), *M. fallax* (670 bp), *M. hapla* (440 bp), and *M. incognita* (615 bp) were used to further analyze the samples using the PCR method.

3 RESULTS AND DISCUSSION

Root-knot nematodes were detected in 12 of 45 samples (26,7%) originated from pepper (*Capsicum annuum* L.), tomato (*Solanum lycopercisum* L.), potato (*Solanum tuberosum* L.), parsnip (*Pastinaca sativa* L.) and ornamental plants callas (*Calla palustris* L.). Three *Meloidogyne* species (*M. incognita, M. hapla, M. arenaria*) were determined on 12 locations of Horgoš, Sombor, Apatin, Čelarevo, Vojka, Pećinci, Glogonj, Crepaja in 5 districts (North Bačka, South Bačka, West Bačka, Srem district, South Banat). *Meloidogyne* species were the most common in pepper, then in tomato and callas originated from greenhouses (9 samples). Three samples originated from potato and parsnip crops were infected with RKN. *Meloidogyne incognita* were detected in 9 samples (5 on pepper, 2 on tomato, 1 on potato, 1 on calla), *M. hapla* (2 samples on parsnip) and *M. arenaria* (1 sample on calla). For all 9 *M. incognita* populations, a PCR was conducted using 194/195 and Mi-F/Mi-R and DNA band of 999 bp was obtained. Two *M. hapla* populations were identified using primers 194/195 and JMV1/JMV2 hapla /JMV tropical and a 700 bp DNA bands were obtained. The primers 194/195 and Far/Rar produced 420bp DNA bands for only one *M. arenaria* population. Comparing the results of this survey with information on occurrence of *Meloidogyne* species detected in Vojvodina Province in the past, the same locations (Horgoš, Crepaja) were mentioned in the literature. According to Jovičić and Grujičić (1986) and Bačić *et al*. (2016, 2022a, 2002b), *Meloidogyne incognita, M. hapla* and *M. arenaria* were found on sugar beet (*Beta vulgaris* L.), potato crops and calla in Vojvodina Province.

4 CONCLUSIONS

The results showed that *M. incognita* is the dominant RKN species in Vojvodina province in Serbia. The climate change and increase of temperatures indicate that *M.*

incognita will cause significantly greater damage in potato production in the future and may become a phytosanitary problem for the Balkan region and possibly for other temperate regions as well. The correct identification of RKN species is the first step towards efficient pest control that ensures healthy plants and good yields. Therefore, a national survey of RKN in Serbia is also planned for the future.

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