Full Length Research Paper

Identification of *Pratylenchus* spp. in soybean in Central region of Brazil using the ITS-5.8S rDNA region

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Root-lesion nematodes are a serious yield-reducing disease of many crops in many parts of the world. The aim of this study was identify populations of *Pratylenchus* spp. in different soybean fields in the Central region of Brazil based upon sequence analyze of ITS-5.8S rDNA region and comparison on GenBank by BLAST. The results showed that a total of 10 nematodes populations showed similarity with *Pratylenchus brachyurus* and remainder showed similarity *Pratylenchus bolivianus*. This is the first report that could indicate the occurrence of mixed populations and a new species of the genus *Pratylenchus* in soybean crops in Brazil.

Key words: Polymerase chain reaction (PCR), *Pratylenchus bolivianus*, *Pratylenchus brachyurus*, ITS region, soybean.

INTRODUCTION

Root-lesion nematodes are economically important migratory endoparasites. *Pratylenchus* sp. are widely distributed throughout the tropical, subtropical and temperate regions of the world (Jatala and Bridge, 1990). They cause local lesions on young roots, and suppress root growth, causing significant yield losses (Castillo and Vovlas, 2007). In Brazil, *P. brachyurus* is becoming increasingly significant in soybean (*Glycine max*) fields, especially in the Central region. In the state of Mato Grosso its frequency reached 96%, higher than that of other nematodes important to the culture as *Heterodera glycines* (35%), *Meloidogyne* spp. (23%), and *Rotylenchus reniformis* (4%), often associated with significant damage to the crop (Silva et al., 2004). It is known that nematode populations in different regions of Brazil can exhibit variations in pathogenicity in a given host, as has already occurred with *Pratylenchus coffeae* (Kubo et al., 2003). The identification of nematodes species has been based only on morphological and morphometric nematode studies. However, molecular characterization has already revealed cases of erroneous identification and the presence of other species in the studies of *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus* spp. and *Ditylenchus* spp. (De Waele and Eisen, 2007). Therefore, the aim of this study was to identify *Pratylenchus* species that occur in soybean fields in the Central region of Brazil using Polymerase chain reaction (PCR) and analyzing nucleotide sequences in the ITS-5.8S rDNA region.

MATERIALS AND METHODS

A total of 12 nematode populations were obtained from soybeans field in different regions of Central region of Brazil (Table 1).
Table 1. Isolates of *Pratylenchus* spp. from the Western Central Brazil used to compare the genetic similarity with sequences of *P. brachyurus* e *P. bolivianus*.

<table>
<thead>
<tr>
<th>Identification of isolate</th>
<th>Geographical origin city (state)</th>
<th>Species</th>
<th>Identity of sequence GenBank (%)</th>
<th>Access number*</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Montidiviu (GO)</td>
<td><em>P. brachyurus</em></td>
<td>81</td>
<td>FJ712900</td>
<td>Waeyenberge et al. (2009)</td>
</tr>
<tr>
<td>02</td>
<td>Rio Verde (GO)</td>
<td><em>P. brachyurus</em></td>
<td>83</td>
<td>HQ662585</td>
<td>Zhao (2010a)</td>
</tr>
<tr>
<td>03</td>
<td>Montidiviu (GO)</td>
<td><em>P. brachyurus</em></td>
<td>84</td>
<td>HQ641384</td>
<td>Zhao (2010b)</td>
</tr>
<tr>
<td>04</td>
<td>Rio Verde (GO)</td>
<td><em>P. brachyurus</em></td>
<td>92</td>
<td>HQ662583</td>
<td>Zhao (2010a)</td>
</tr>
<tr>
<td>05</td>
<td>Chapadão Céu (GO)</td>
<td><em>P. brachyurus</em></td>
<td>77</td>
<td>HQ662583</td>
<td>Zhao (2010a)</td>
</tr>
<tr>
<td>06</td>
<td>Mineiros (GO)</td>
<td><em>P. bolivianus</em></td>
<td>84</td>
<td>HM469447</td>
<td>Wang et al. (2010)</td>
</tr>
<tr>
<td>07</td>
<td>Paraúna (GO)</td>
<td><em>P. brachyurus</em></td>
<td>81</td>
<td>FJ712898</td>
<td>Waeyenberge et al. (2009)</td>
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<tr>
<td>08</td>
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<td>78</td>
<td>FJ712893</td>
<td>Waeyenberge et al. (2009)</td>
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</tr>
<tr>
<td>10</td>
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<td>93</td>
<td>FJ712900</td>
<td>Waeyenberge et al. (2009)</td>
</tr>
<tr>
<td>11</td>
<td>Querência (MT)</td>
<td><em>P. brachyurus</em></td>
<td>71</td>
<td>HQ641384</td>
<td>Zhao (2010b)</td>
</tr>
<tr>
<td>12</td>
<td>Querência (MT)</td>
<td><em>P. brachyurus</em></td>
<td>74</td>
<td>HQ641385</td>
<td>Zhao (2010b)</td>
</tr>
</tbody>
</table>

*Accession number in GenBank - National Center of Biotechnology Information (NCBI).*

From Goiás state were obtained two isolates in the municipality of Montidiviu, three isolates in the municipality of Rio Verde and one isolate in the municipalities of Mineiros, Chapadão do Céu and Paraúna. From Mato Grosso state were obtained two isolates in the municipality Querência (Figure 1).

The nematode populations were kept in a greenhouse on common bean (*Phaseolus vulgaris* L.) cv. Jalo for six months. The nematodes were extracted from common beans roots using the method described by Coolen and D’Herde (1972). The total DNA of twenty nematodes (Troccoli et al., 2008) from each population was extracted using the "PureLink™ Genomic DNA Mini Kit" (Invitrogen®) following the procedure described by the manufacturer (Invitrogen Kit Manual). The primers ITS4 (5′-TCCTCGGGTATGTGATAT-3′) and ITSS (5′-GGAGTAAAGCTTGACACCAT-3′) (White et al., 1990) were used for amplification of the ITS-5.8S rDNA region. The PCR products were analyzed by electrophoresis in agarose gel 2%.

The PCR products directed for DNA sequencing were purified with the ExoZap (USB corporation). The sequencing analysis was carried out at the Center for Human Genome Studies Center at the University of São Paulo. The DNA sequences were analyzed using the Bioedit 7.0.9 program (Hall, 1999) and compared on GenBank by BLAST searches (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi).

RESULTS AND DISCUSSION

Sequencing of the ITS-5.8S rDNA region fragment resulted in nucleotide information of approximately 800 bp, for all isolates listed in Table 1. Through BLAST searches a total of 10 nematodes populations showed similarity (71 to 93%) with *P. brachyurus*, among nematode species with information available on GenBank. The population 11 and 12 showed similarity (78 and 84%, respectively) with *P. bolivianus* (Table 1). This percentage of similarity (genetic identity) is comparable to the degree of similarity between individuals of the same species and it is used for identifying nematode species (Oliveira et al., 2009). *P. bolivianus* has been reported in the USA in roots of *Erica* sp. In South America, this species has been related in oat and potato roots in Bolivia and also in the tundra regions of Chile (Castillo and Vovlas, 2007). In root of *Alstroemeria* in United Kingdom (Waeyenberge et al., 2000).

Identification of root-lesion nematodes is difficult due to overlap of morphometric characters and little morphological diversity (Roman and Hirschmann, 1969; Tarte and Mai, 1976). *P. brachyurus* and *P. bolivianus* are morphologically similar, which complicates their identification (Frederick and Tarjan, 1989). Morphological features cannot be separated using optical microscope. According to Tarte and May (1976) and Tarjan and Frederick (1978), the stylet length and relative position of the vulva have been the main morphological characteristics to differ species of *Pratylenchus*. In an attempt to elucidate the genetic variations of this pathogen, several authors have used biochemical techniques to assist in clarifying the existence of variability. Payan and Dickson (1990) showed the possible existence of genetic variability among different populations of *P. brachyurus* acquired from different regions of the United States.

Waeyenberge et al. (2000) showed that there were interspecific variations in 18 species of *Pratylenchus* from different geographical regions. Intraspecific variation in populations of Pratylenchus have also been reported (De Luca et al., 2004). The main causes of this variation are in the presence of repeated sequences and the differences in the size of the ITS rDNA region (Depaquit et al., 2002). For a rapid and correct identification of *Pratylenchus* species should be used a species-specific
primers, however, there are a few primers developed for it. Al Banna et al. (2004) developed specific primers for Pratylenchus species, except for P. brachyurus, because this species exhibits variations in the sequences of the region D3. Machado et al. (2007) suggested the presence of heterogeneity in copies of the ITS region within the individuals of P. brachyurus. This is the first report of the possible existence of mixed populations or the occurrence of a new species of the genus Pratylenchus in soybean crops in Brazil. However, P. brachyurus and P. bolivianus are very similar in morphological terms (Frederick and Tarjan, 1989). Because of the difficulty in distinguishing the two species using conventional methods, molecular techniques will become increasingly important to quickly and accurately identify these nematode species. It is essential that nematodes are correctly identified for integrated management strategies. The kit for extraction of DNA used in this study was not possible to extract sufficient DNA for subsequent molecular analysis, such as the study of the phylogenetic relationship between species Pratylenchus spp. This research project should provide support for future studies to elucidate the variations in Pratylenchus populations in Brazil, and reinforce the need for pathogenicity studies so that effective control strategies can be developed.

REFERENCES

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