MOLECULAR IDENTIFICATION OF A DESTRUCTIVE PHYTOPATHOGENIC FUNGUS IN TOMATO FIELDS OF IRAN

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Abstract:

The necrotrophic fungus *Rhizoctonia solani* is one of the most important, soil-borne pathogens with a wide host range. This pathogen is the casual agent of crown rot, root rot and damping off in tomato producing areas. The purpose of this research was to characterize the *Rhizoctonia* spp. isolates obtained from tomato at different taxonomic levels, to investigated genetic diversity and pathogenicity of these isolates Sampling was carried out in the main tomato growing regions of Khorassan-Razavi Province during 2011-2012. Morphological characteristics such as the hyphal diameter and conformation, presence of dolipore septum, the number of nuclei per hyphal cell, the size of monilioid cells and sclerotia were investigated for the 39 *Rhizoctonia* spp. isolates obtained from tomato plants. Additionally, 7 *R. solani* isolates obtained from potato were investigated in this research. In total, 46 *Rhizoctonia* spp. isolates were obtained which consisted of 41 *R. solani* isolates and 5 isolates belonged to binucleate *Rhizoctonia* spp. Morphological characterization revealed variability among the isolates. The anastomosis grouping on slide was primarily used for determining anastomosis groups (AGs) and intraspecific groups (ISGs). The PCR-RFLP method was used to confirm the identified AGs and determine the ISGs. In this method. The ITS1, 5.8S, and ITS2 regions of rDNA were amplified using the species-specific primers of *R.solani* including RS1 and RS4. Then the PCR product was restricted using various restriction enzymes such as *Mun* I, *Mse* I, *Hinc* II and *Ava* II. The AG and ISG of each isolate was determined by comparing the restriction pattern of each *R. solani* isolate obtained in this study with the pattern of standard isolates. The PCR- RFLP analysis revealed that *R. solani* isolates belonged to AG2-1, AG3 PT, AG4 HG I, and AG4 HG II with the number of 4, 15, 13, and 9 isolates respectively.

Key Words: Molecular Identification, Destructive Phytopathogenic Fungus, Tomato Fields

Introduction

Tomato foot and root rot, caused by Rhizoctonia solani (teleomorph: Thanatephorus cucumeris), is one of the most destructive diseases in some of the tomato production areas worldwide [1,2]. To date, this fungal species is subdivided into 14 anastomosis groups (AGs) designated as AG 1 through 13 and bridging isolate (BI) group [3,4]. Several AGs of R. solani such as AG 2-1 [5], AG 3 [5,6], and AG 4 HG I [2,7] have been shown to be pathogenic on tomato, the most frequently reported being AG 3. Knowledge about the prevalence and distribution of different AGs is important, since sensitivity to chemical control treatments and probably to other control strategies is varying among AGs [8]. In addition to the similarity of disease symptoms, distinguishing the various *Rhizoctonia* species in culture is difficult due to the lack of stable morphological characters on which to base a definitive classification of the genus *Rhizoctonia* and species assigned to it. Also, identification of the intraspecific groups of various AGs based on anastomosis grouping on a slide is not accurate.

Foot rot symptoms can be characterized by soft rot of the seedlings near the soil surface. Because of the high variability in the Rhizoctonia populations, its wide host range, and living as a soil borne pathogen with long-term survival in soil, management of diseases caused by this fungus is difficult [9].

The objectives of the present study was to characterize *Rhizoctonia* spp. associated with tomato diseases in Iran by morphological characterization and hyphal anastomosis in conjunction with molecular techniques.

Materials And Methods

Fungal isolates. Samples from each of the geographically defined tomatorice-growing areas in Iran were collected using transect sampling by walking through the field diagonally. Per field, 10 samples were collected all along the path of the diagonal. Plant tissues of tomato root, crown and leaves with the disease symptoms were surface-disinfected with 0.5% sodium hypochlorite solution for 2 min and rinsed three times with sterile distilled water. Then, pieces of sheath or leaf blade were dried on sterilized filter paper, placed on a petri dish containing acidified water agar (PH 4.5) with 10% lactic acid (AWA), and incubated at 28°C in the dark. After 2 to 3 days, cultures were examined microscopically for hyphal characteristics typical of *Rhizoctonia* spp. . All plated samples readily yielded *Rhizoctonia* spp., and a hyphal tip of each isolate was subcultured onto AWA for further purification. Isolates were transferred to potato dextrose agar (PDA) test tube slants and maintained at 28°C. Following sufficient growth and production of sclerotia, culture tubes were kept at 4°C for short-term storage. Tester strains of *R. solani* were used for AG typing on glass slides. For long-term storage of the isolates, one of the following methods was used: (i) culturing the fungus on sterile barley grains or (ii) storing lyophilized fungal mycelium or sclerotia at 4°C.

DNA extraction. Cultures were grown at room temperature without shaking in 125-ml Erlenmeyer flasks containing 75 ml of potato dextrose broth (PDB), 24 g/liter (Difco Laboratories, Detroit, MI). Mycelium was harvested after 5 days, preceding sclerotium formation. Lyophilized tissue was pulverized in liquid nitrogen. Total genomic DNA was extracted by the hexadecyltrimethylammonium bromide method according to Zolan and Pukkila (46). Total DNA was dissolved in 50 to 200 µl of Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) depending on the size of the DNA pellet, and quantified by spectrophotometry. Dissolved DNA was stored at –20°C until used.

PCR-RFLP of rDNA-ITS region. RFLP of rDNA-ITS was employed to clarify whether isolates of *R. solani* AG1 obtained from rice belonged to subgroup IA, IB, or IC. Tester strains of AG1-IA (CSKA) obtained from rice, and of AG1-IB (B19) and AG1-IC (BV17), both obtained from sugar beet, were included for comparison. Genomic DNA of the isolates was used for PCR amplification of the ITS region using a pair of primers, RS1 (5′-CCTGTGCACCTGTGAGACAG-3′ and RS4 (5′-TGTCCAAGTCAATGGACTAT- 3′), using the reaction conditions described by Guillemaut et al. (10). Restriction polymorphisms were detected using two discriminating restriction enzymes, *Mse*I and *Mun*I (MBI Fermentas, Vilnius, Lithuania) (10). Typical restriction enzyme reactions consisted of 7 µl of PCR product, 10 units of restriction enzyme, 2 µl of reaction buffer, 0.2 µl of bovine serum albumin, and H2O to a total volume of 20 µl. Reactions were incubated for 3 h at 37°C (or according to manufacturer's recommendations), then stopped by incubation at 70°C for 5 min.

Results and Discussion

The rDNA-ITS region, including ITS1, 5.8S, and ITS2 for 99 isolates of *R. solani* and the tester isolates of R. solani was amplified and digested using two discriminating restriction enzymes (*Mun*I and *Mse*I) (Figures 1 and 2). The three ISGs of *R. solani* AG4 (HG I and HG II) differed from each other in restriction sites generated with restriction enzymes used in this study. The smaller bands were 0.12 and 0.06 kb for the *R. solani* isolates, respectively. In both cases (digestion with *Mun*I or *Mse*I), restriction fragments less than 0.05 kb were not taken into consideration because they would not be clearly resolved by electrophoresis. The whole PCR-RFLP procedure was repeated twice with similar results and it was capable of separating the Rhizoctonia isolates belonging to various taxonomic groups.

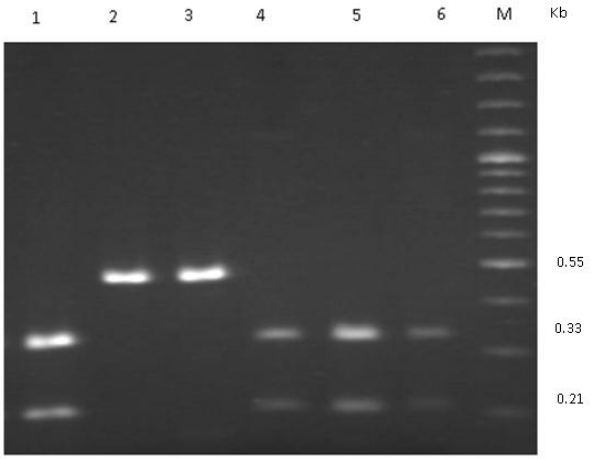

Figure 1. Restriction pattern of *Mun* I

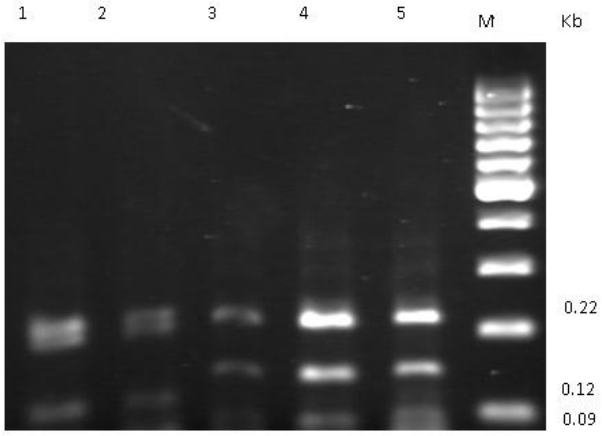

Figure 2. Restriction pattern of *Mse* I

References:

[1] Solanki MK, Robert AS, Singh RK, Kumar S, Pandey AK, Srivastava AK, et al. Characterization of mycolytic enzymes of Bacillus strains and their bio-protection role against Rhizoctonia solani in tomato. Curr Microbiol 2012. http://dx.doi.org/10.1007/s00284-012-0160-1.

[2] Taheri P, Tarighi S. The Role of pathogenesis-related proteins in tomato-Rhizoctonia solani interaction. J Bot 2012. http://dx.doi.org/10.1155/2012/137037.

[3] Carling DE, Kuninaga S, Brainard KA. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of Rhizoctonia solani anastomosis group-2 (AG-2) and AG-BI. Phytopathology 2002;92:43e50.

[4] Sneh B, Burpee L, Ogoshi A. Identification of Rhizoctonia species. St. Paul, MN: The American Phytopathological Society Press; 1991.

[5] Misawa T, Kuninaga S. The first report of tomato foot rot caused by Rhizoctonia solani AG-3 PT and AG-2-Nt and its host range and molecular characterization. J Gen Plant Pathol 2010;76:310e9.

[6] CharltonND, CarboneI Tavantzis SM, Cubeta MA. Phylogenetic relatedness of the M2 double-stranded RNA in Rhizoctonia fungi. Mycologia 2008;100:555e64.

[7] Kuramae EE, Buzeto AL, Ciampi MB, Souza NL. Identification of Rhizoctonia solani AG 1-IB in lettuce, AG 4 HG-I in tomato and melon, and AG 4 HG-III in broccoli and spinach, in Brazil. Eur J Plant Pathol 2003;109:391e5.

[8] Campion C, Chatot C, Perraton B, Andrivon D. Anastomosis groups, pathogenicity and sensitivity to fungicides of Rhizoctonia solani isolates collected on potato crops in France. Eur J Plant Pathol 2003;109:983e92.

[9] Taheri P, Tarighi S. Riboflavin induces resistance in rice against Rhizoctonia solani via jasmonate-mediated priming of phenylpropanoid pathway. J Plant Physiol 2010;167:201e8.

[10] Guillemaut C, Herman VE, Camporota P, Alabouvette C, Molard MR, and Steinberg C. Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA. Can. J. Microbiol 2003;49:556-568.