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Full Length Research Paper

First morpho-molecular identification of *Rhizoctonia solani* AG-7 from potato tuber-borne sclerotium in Saudi Arabia

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Losses in yield and potato tubers quality caused by *Rhizoctonia solani* in Saudi Arabia was investigated in 2007. A number of sclerotia of *R. solani* were isolated from potato tubers collected from Saudi Arabia. Based on morphological observations, hyphal fusion compatibility and pathogenicity tests, most isolates collected belonged to AG 3, but 13, 5 and 2% of the isolates were AG-4, AG-5 and AG-7, respectively. What is more, hyphal branching at a right angle with a constriction and septum near the origin, multinucleate and monilioid cells and hyphal diameter were investigated. Cultural appearances of *R. solani* AG-7 growing on malt extract agar (MEA), dextrose sabroud agar (SDA), Dox agar (DOX), potato-dextrose agar (PDA) were brown or dark brown colony with aerial mycelia and sclerotia, but without clear zonation on DOX and PDA. Culture of AG-7 had abundant pitted sclerotia with dark brown exudates on SDA. Currently recovered isolates of *R. solani* shows hyphal anastomosis with AG-7 tester isolates. Koch's postulates were applied by re-isolation of the fungus from inoculated tubers. The microsatellite-primed polymerase chain reaction (MP-PCR) was used to identify *R. solani* AG-7. The reference laboratory tester and *R. solani* isolates (AG-7) produced highly homologous fingerprints (level of homology, 86%). By comparing specific MP-PCR fingerprints of unidentified *R. solani* isolates with those of AGs testers, *R. solani* isolates (AG-7) could be identified to the group level even if they could not be identified by routine morphological methods. This is claimed to be the first report of *R. solani* AG-7 in Saudi Arabia.

Key words: AG-7, anastomosis typing, potato, sclerotia, *Thanatephorus cucumeris*.

INTRODUCTION

Potato (*Solanum tuberosum*) is one of the most important crops cultivated for human foods and trade worldwide (Potato News, 2004). *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) was first reported on potato tubers (Kühn, 1858). Recently, *R. solani* has developed into an important pathogen in potato growing-areas in Saudi Arabia (El-Hussieni, 2004).

Rhizoctonia can delay emergence, reduce stands and in some cases, limit yields (Errampalli and Johnston, 2001) ultimately reducing tuber quality and therefore, marketability. Black scurf disease cause significant economic losses annually, but very rarely do they cause losses in any one field or in a single season (Banville, 1989; Krechel et al., 2002). Isolates of *Rhizoctonia* species have been placed further into anastomosis groups (AGs) based on their hyphal behavior. Hitherto, at least 13 anastomosis groups have been reported (Woodhall et al., 2007). *R. solani* anastomosis group AG-3 is a common fungal inhabitant of the Potato tuber rhizosphere and has a universal distribution (Cubeta and Vilgalys, 2000). *R. solani* AG-3 represents the most genetically tractable member of the *R. solani* species complex and is a pathogen that affects important food crops in the plant family Solanaceae, including eggplant, pepper and potato (Ceresini et al., 2007). The diseases caused by AG-3 on potato are known as stem canker and black scurf, which occur in all potato-growing areas world-

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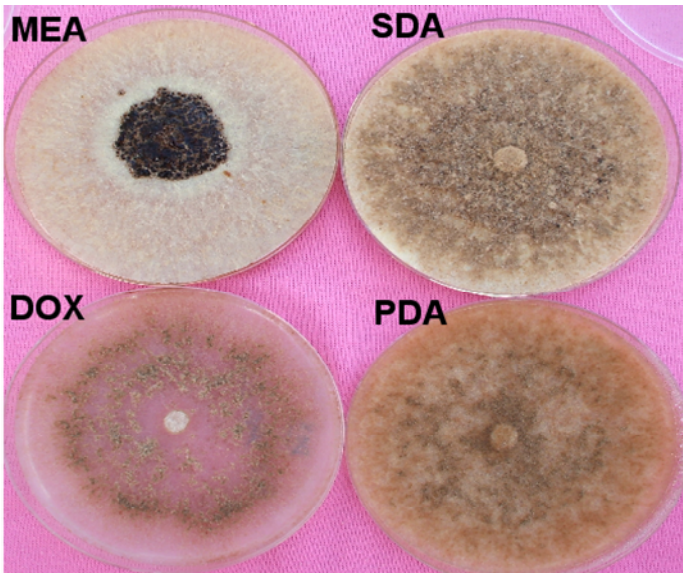

Figure 1. Mycelial and sclerotial growth of *R. solani* AG-7 on four different types of media (MEA, SDA, DOX and PDA).

wide (Lehtonen et al., 2008). The predominant isolates found to affect potato plants belong to anastomosis group AG-3 (Carling and Leiner, 1990; Ritchie et al., 2006). Anastomosis studies indicated that potato isolates belonged to AG-3 and AG-5 groups in India (Thind and Aggarwa, 2008). Previously, *R. solani* AG-7 has been recorded on tubers in potato-growing areas in different countries (Carling et al., 1998; Zakria et al., 2002; Truter and Wehner, 2004). The aim of this work was to identify *R. solani* anastomosis group collected from potato tubers and also the application of PCR fingerprinting was performed using three sets of primers (AG)8C, $(AGG)_5$ and $(CAG)_5$ for the purpose of *R. solani* anastmosis groups (AG-7) identification.

MATERIALS AND METHODS

R. solani isolates were recovered from superficial tuber alterations, like deformations, corky or scabby lesions. Infected tubers were washed for 2 min with tap water, surface-sterilized in sodium hypochlorite (1%) for 2 min and washed in 4 changes of sterilized tap water and blotted dry on absorbent paper. Visible sclerotia or the mature mycelia found on tubers with the aid of a binocular microscope were placed on PDA and incubated at room temperature (26°C). Hyphal tips from subsequent growth were transferred to potato dextrose agar (PDA) after 24 h to isolate *R. solani* AG-3. The isolates were stored on dried cereal grains (Sneh et al., 1991).

R. solani isolates were identified morphologically by examining the hyphal branching, the septal pore type and the number of nuclei per cell after 2 days of growth on Water Agar (WA) followed by staining with aniline blue. Also, sclerotium production and monilioid cells were shacked after 14 days growth on potato dextrose agar (PDA). Anastomosis grouping was determined by the technique reported by Balali et al. (1995). Hyphal interactions were assigned to one of the four categories in accordance with those described by MacNish et al. (1997). Pathogenicity assessment of *R. solani* isolates were done by toothpick inoculation of tuber. The pathogenicity of tuber-borne inoculum was verified by growing plants from sclerotia-infested tubers (Zakria et al., 2002). Total DNA was extracted as described by Abd-Elsalam et al. (2007). PCR amplification was performed in Peltier Thermal Cycler-200 (MJ Research Inc.). Polymerase Chain Reaction were performed using 50 ng of template DNA in 25 µL reaction volume using 0.2 unit of Taq polymerase with reaction buffer, containing buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$,10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100. pH 8.8). The PCR program consisted of 1 cycle of 2 min at 94°C followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 52 °C for 90s and extension at 72 °C for 2 min. The thermal profile ended with a final extension at 72°C for 6 min. Following amplification, the samples were separated by electrophoresis in 1.5% agarose gel, stained with 0.5 µg/ml of ethidium bromide and viewed under ultra-violet light. All MP-PCR patterns were analyzed with Fingerprinting Software (Gel Documentation and Analysis Systems, Uvitec, Cambridge, UK.). Bands were automatically identified by the resident software, verified and edited manually. Dendrograms were generated by the hierarchic unweighted pair-group method with arithmetic averages (UPGMA) cluster algorithm.

RESULTS AND DISCUSSION

The morphology of the isolates was significantly diverse. Consequently, the isolates were classified into 4 anastomosis groups. Of the 100 multinucleate isolates of *R. solani* tested, 80% were AG-3, 13% were AG-4, 5% were AG-5 and 2% were AG-7. Symptom-less tubers yielded two AG-3 isolates and two AG-4 isolates. These results showed that, although AG 3 (potato type) is the most common *R. solani* group on potato in Saudi Arabia, AG-4 and AG-7 may also be present. Colonies of AG-7 isolates grown on malt extract agar (MEA), dextrose sabouraud agar (SDA), Dox agar (DOX), potato-dextrose agar (PDA) were brown to dark brown with aerial mycelium and sclerotia. The isolates had pitted sclerotial clusters and brownish exudates after 21 days of culturing on SDA, but without clear zonation.

AG-7 had aerolate hyphae to woolly tufts of mycelium after three weeks growth on PDA. Also AG-7 had bumpy sclerotial clusters and brownish exudates after three weeks growth on malt extract agar (MEA) medium. Superior sclerotial formation was observed on all tested media (Figure 1). Sclerotia and moniliod cells were observed on all tested media (Figures 2a and b). All the isolates were multinucleate and shared distinctive characteristics with *R. solani*. Young mycelium showed right-angled branching and constriction near each branch (Figure 2c); older mycelium with barrel-shaped cells (Figure 2d).

The symptoms of the disease were found below ground portions of the plant. Fully-grown tubers showed the characteristic thin layers of dark brown scurf and asymmetrical bulges (sclerotia) of the fungus. These resting bodies were up to 6 mm across and adhere closely to the skin on the tuber (Figure 3). Reisolation from inoculated plants yielded colonies that were anastomosed with both AG-3 and AG-7 tester isolates. *R. solani*

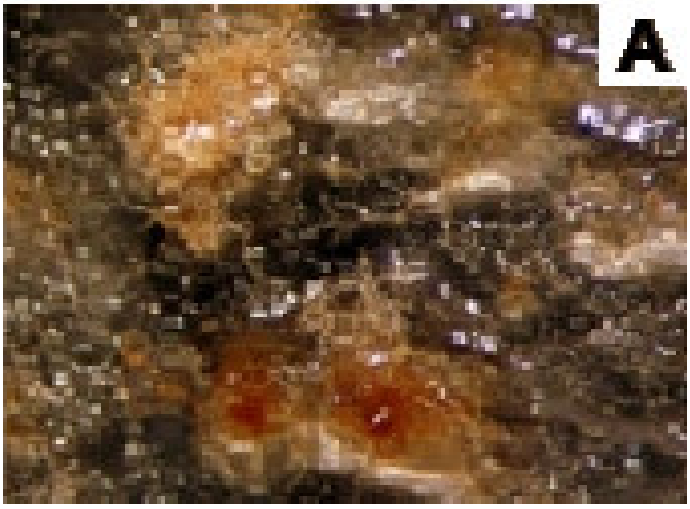

Figure 2a. Cultural characteristics of *R. solani* AG-7; 'micro-sclerotial' type of sclerotial formation among entangled hyphae;

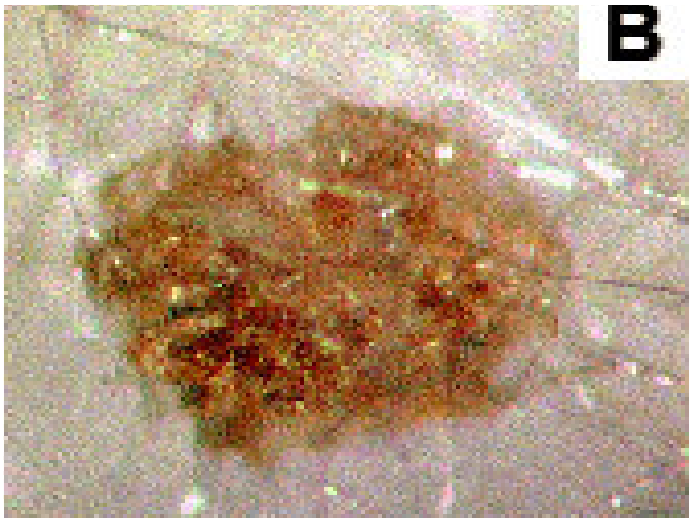

Figure 2b. Cultural characteristics of *R. solani* AG-7; irregular sclerotia, brown at maturity;

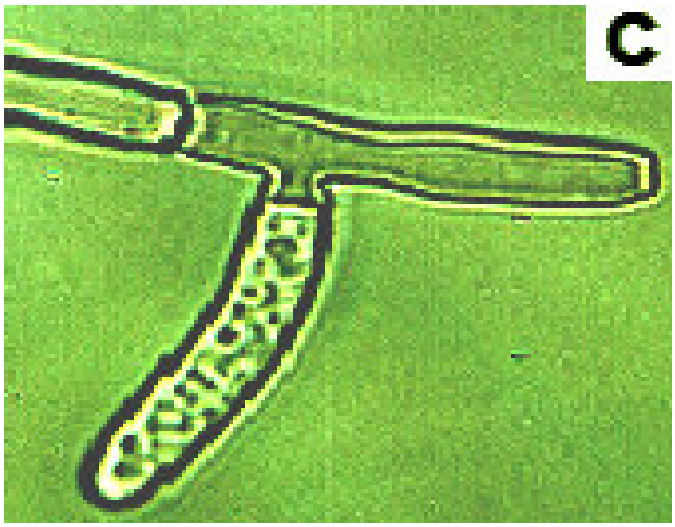

Figure 2c. Cultural characteristics of *R. solani* AG-7; Septa and nuclei in vegetative hyphae of mulinucleate species (400 X).

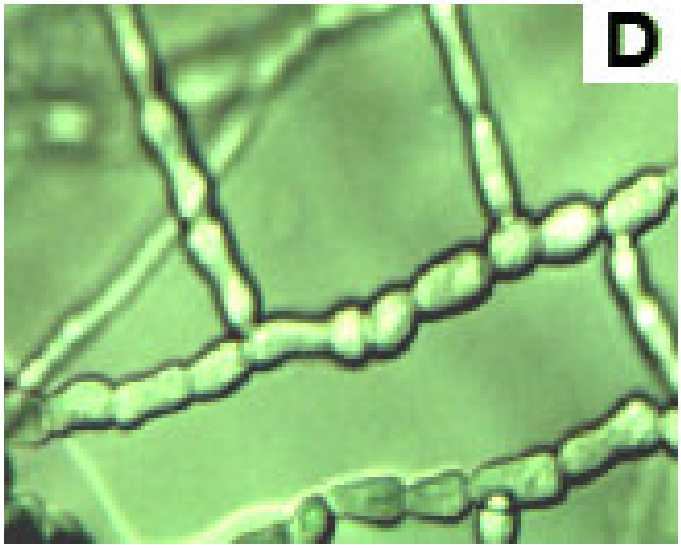

Figure 2d. Cultural characteristics of *R. solani* AG-7; barrel-shaped moniliod cells in chain, 14 days at 28℃ on PDA (400 X).

solani AG-3 is relatively specific to potato, sclerotia on tubers belong almost exclusively to AG-3. *R. solani* isolates of the anastomosis group AG-3 and AG-7 were more virulent than other AGs (Carling et al., 1998; Zakria et al., 2002). While, none of the AG-7 and AG-8 isolates showed any virulence to potato sprouts (Truter and Wehner, 2004). During *in vivo* tests, most of the AG-3 isolates were significantly more virulent than isolates belonging to other AGs. Three microsatellite tandem repeated di and trimers, (AG) 8C, (AGG)5 and (CAG)5, were used for *R. solani* anastomsis groups differentiation. Interestingly, the combined dendrogram of three MP-PCR primers displayed high similarity (86%) between RS1, RS2 and AG-7 isolates (Figure 4). The three microsattlite primers not only revealed regional polymorphic specific bands but analysis of their sum band profiles correctly categorized *R. solani* anastomosis groups. The discriminating powers of the three primers used in this study were nearly the same. According to the results presented in dendrogram, to identify the unknown isolate at species level, independent MP-PCR analyses with at least three different primers should be performed and compared to the results for anastomosis group typing. These findings suggest that MP-PCR is a reliable technique because microsatellites are considered to be high-resolution markers.

Figure 3. Black scurf of potato tubers caused by *R. solani*.

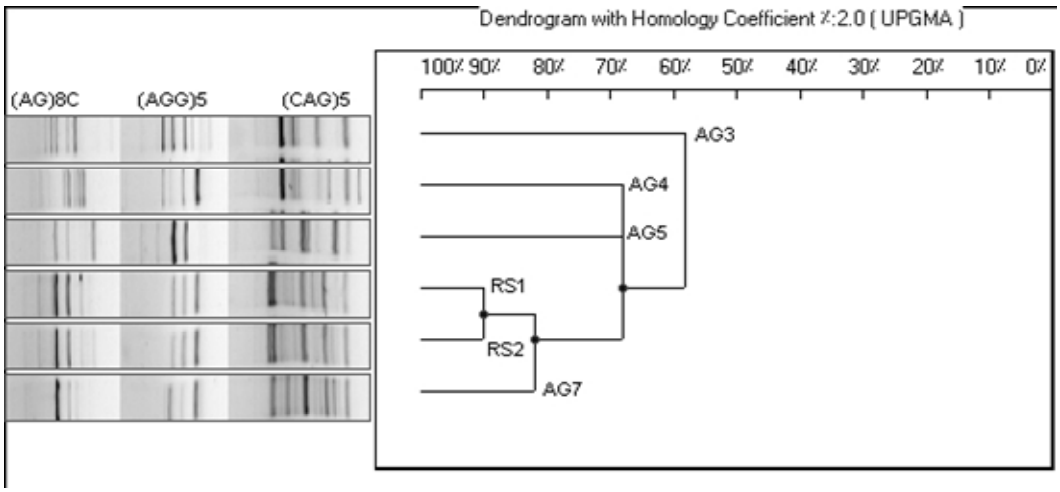

Figure 4. The microsatellite UPGMA dendrogram for three combined primers of *R. solani* isolates constructed using Pearson correlation coefficient, respectively. The microsatellite allele present in one isolate was scored as (1) and its absence in another as (0).

The use of co-dominant MP-PCR markers enables the analysis of fragments with a high reliability and to detect sufficient intra-specific variation for the tested *R. solani* anastomosis groups (Guleria et al., 2007; Mwang'Ombe et al., 2007; Abd-Elsalam et al., 2009). Our findings suggested that rhizoctonia disease in potato fields in Saudi Arabia is caused by a mixture of disparate anastomosis groups. These results will be imperative for controlling black scurf disease in Saudi Arabia.

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