African Journal of Microbiology Research Vol. 6(37), pp. 6661-6667, 27 September, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.1096
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Diversity among some Egyptian isolates of *Rhizoctonia solani* based on anastomosis grouping, molecular identification and virulence on common bean

Rashad Y. M.[1]*, Abdel-Fattah G. M.[2], Hafez E. E.[3] and El-Haddad S. A.[4]

[1]Science Department, Teachers College, King Saud University, Saudi Arabia.
[2]Plant Production Department, College of Food and Agricultural Sciences, King Saud University, Saudi Arabia
[3]City for Scientific Research and Technology Applications, Arid Lands Research and development institute, New Borg El-Arab, 21934, Alexandria, Egypt.
[4]Mycological Research and Disease Survey Department, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

Accepted 17 September, 2012

Nine isolates of *Rhizoctonia solani* were obtained from different plant types (common bean, broad bean, bell pepper, tomato and cucumber). The obtained isolates were assigned to AG according to hyphal anastomosis. Of these, six isolates belonged to AG 2-2 IIIB, while the other three isolates belonged to AG 4 HG-I. Molecular identification using 18S-rRNA gene showed that all the isolates were *R. solani* with sequence identity 99% which revealed that these isolates comes from one ancestor, but the analysis based on rRNA-18S sequences failed to group them into different distinct groups on the bases of AGs. Pathogenicity test on common bean under greenhouse conditions showed that all isolates have the potency to cause seed rot, pre-emergence, post-emergence damping-off and root rot diseases, where they caused mortality ranged from 13.33 to 100%. On the other hand, four bean cultivars were tested (Giza 3, Giza 6, Contendor and Rajma). All tested bean cultivars manifested the disease symptoms but Giza 3 was the most susceptible one. It showed the highest value of total mortality (94.07%).

Key words: Anastomosis, common bean, damping-off, 18S rRNA, root rot.

INTRODUCTION

Rhizoctonia solani Kühn (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a soil-borne fungus that causes seed decay, damping-off, stem canker, root rot, fruit decay and foliage diseases in many crop species including common bean (*Phaseolus vulgaris* L.) (Sneh et al., 1996). This unlimited host range, combined with competitive saprophytic ability and lethal pathogenic potential, earn *R. solani* its status as formidable pathogen. *Rhizoctonia* root rot disease is a serious and economically important disease for bean production in most of the tropical, subtropical and temperate areas of the world where it is grown (Tu et al., 1996). Yield losses of 5 to 10% are common, but 60% yield losses have been reported in Brazil.

R. solani is called a species complex because it contains many related but genetically isolated sub-specific groups. Because *R. solani* and other *Rhizoctonia* fungi do not produce conidia and only rarely produce basidiospores, the classification of these fungi has often been difficult. Current classification within the *R. solani* species complex relies largely on the grouping of isolates into anastomosis groups (AG) based on hyphal interactions (Ceresini et al., 2007). To date, isolates of *R. solani* have been assigned to 14 AGs, including AG-1 to AG-13 and AG-B1 (Carling et al., 2002). While four of the fourteen AGs are not pathogenic (AG6,7,10 and AG BI), four (AG-1, -2, -3, and -4) cause important diseases on plants worldwide; and the remaining AGs are less destructive pathogens with generally more restricted

*Corresponding author. E-mail: younesrashad@yahoo.com. Fax: +966 4915684. Tel: +966 4911063

geographic distributions (Carling et al., 2002). Of 229 *R. solani* isolates obtained from bean plants and soils in Samsun province, 59% of the isolates belonged to anastomosis group AG 4, 31% to AG 2-2 and the remaining 10% to AG 5. The virulence of the isolates on different bean cultivars varied. AG 4 and AG 2-2 group isolates caused severe symptoms of root rot on all cultivars. AG 5 isolate was highly to moderately virulent regarding the susceptibility of the bean cultivars (Karaca et al., 2002). Muyolo et al. (1993) found that of 290 *R. solani* isolates, all AG-1 IB isolates caused foliar blight and root and hypocotyl rot in virulence tests on dry bean. AG-2-2 IIIB isolates were more virulent on roots than on hypocotyls; AG-4 isolates were more virulent on hypocotyls than on roots. Isolates within AG tend to have similar host ranges; according to their morphology and pathogenicity, they have been classified into subgroups: four subgroups of AG-1, eight of AG-2, three of AG-4, two of AG-6, five of AG-8, and two of AG-9 have been reported (Carling, 2000; Priyatmojo et al., 2001; Fenille et al., 2002). Molecular approaches based on the analysis of ribosomal DNA (rDNA) sequences have added genetic support to the AG classification system and allowed the investigation of their evolutionary relationships (Guillemaut et al., 2003). Sequence data may support genetic groups within *Rhizoctonia* species better than other characters used in the past such as number of nuclei, plant host or morphology (Gonzalez et al., 2006). The aim of this study was to investigate anastomosis grouping, ribosomal DNA (rDNA) sequencing, and virulence of isolates of *R. solani* on common bean.

MATERIALS AND METHODS

Isolation, purification and identification of the pathogen

Naturally diseased plants of different types (common bean, broad bean, bell pepper, tomato and cucumber) exhibiting typical symptoms of root rot disease were collected from various governorates namely Al-Dakahlia, Al-Gharbia, Al-Giza, Al-Sharkia, Damietta and Kafr Al-Sheikh.

For fungal isolation, the collected diseased plant roots were washed carefully under running tap water to remove the adjacent soil particles followed by sterile water, then dried between two filter papers. Using sterilized scalpel, roots were cut into small pieces (1 to 2 cm^2). The pieces were then transferred into 1% hypochlorite solution (disinfectant solution) for 3 min for surface sterilization. Surface sterilized pieces were then washed several times with sterilized water to wash out the remaining disinfectant solution. The pieces were then dried on sterilized filter papers. Using sterilized forceps, plot dried pieces were then transferred into Petri dishes containing potato dextrose agar medium (PDA) supplemented with antibacterial agent (L-chloramphenicol 5 mg/L and streptomycin sulphate 5 mg/L). The dishes were then incubated at 28°C, and then checked for fungal growth two days after planting. Purification of the isolates was done using the hyphal tip technique to obtain them in pure cultures; the detected isolates were then transferred into slant of PDA and kept at 4°C for further studies. Pure cultures of the isolated fungi were identified according to the cultural properties, morphological and microscopical characteristics as described by Sneh et al. (1991).

Anastomosis grouping

Field isolates of *R. solani* were assigned to AG according to hyphal anastomosis with tester isolates from AG 1 to AG 10. The tester isolates used in this study were kindly provided by Dr. Shiro Kuninaga (Health Sciences University of Hokkaido, Japan). According to the slide technique of Kronland and Stanghellini (1988), each isolate was paired with tester isolate of each AG on a clean glass slide (cleaned by dipping in 95% ethanol and wiping dry). Mycelial disks (7 mm diameter) of field isolate and tester isolate growing on PDA were spaced 2 to 3 cm away. The slides were placed on moist filter paper in Petri dishes (20 cm diameter) and incubated at 27°C in the dark until the advancing hyphae from opposite disks overlapped slightly (2 to 3 days). When the hyphae from the two disks overlapped, slides were removed, the excess moisture was wiped from the bottom of the slide, and the disks lifted from the slide. The area of overlap was stained with lactophenol blue solution (Merck) and covered with a 22-mm coverlip and examined microscopically at 100× for hyphal anastomosis and fusion was confirmed at 400×. Anastomosis reactions were grouped into categories in which category C0 is no reaction, C1 is hyphae contact only, C2 is killing reaction which represents a somatic incompatibility response between genetically distinct individuals and C3 is a perfect fusion of hyphal cells between two isolates that indicative of genetic identity or near identity. Anastomosis grouping was assessed positively when C2 or C3 occurred in five sites at least (Carling, 1996).

Molecular identification using 18S-rRNA gene

Extraction of genomic DNA

Cultures of Rhizoctonia isolates for DNA extraction were grown in 50 ml of potato dextrose broth (PDB) supplemented with 250 µg ml^{-1} chloramphenicol for 10 days at 28 ± 2°C without shaking. The mycelia were collected by vacuum filtration and stored at -80°C until use. Genomic DNA was extracted according to Edwards et al. (1991).

Amplification of 18S rRNA gene

Polymerase chain reaction (PCR) was performed in a total volume 25 µl containing 2.5 µl 5X Colorless GoTaq® Flexi Buffer, 2.5 µl 5X Green GoTaq® Flexi Buffer, with 2.5 µl $MgCl_2$, 3 µl dNTPs, 2 µl (10 pmol) primer NS1, 2 µl (10 pmol) primer NS2, 2 µl template DNA and 0.2 µl (5 units/µl) GoTaq® Flexi DNA Polymerase (Promega Corporation, USA). PCR amplification was performed in a thermal cycler (Eppendorf, Thermo Fisher Scientific Inc., USA) programmed for one cycle at 95°C for 5 min.

Then, 34 cycles were performed as follows: 1 min at 95°C for denaturation, 1 min at 55°C for annealing and 1 min at 72°C for elongation. Reaction was then incubated at 72°C for 10 min for final extension. PCR products were analyzed by electrophoresis of a sample (5 µl) in horizontal agarose gels, and the size of products was verified by comparison with a molecular weight marker (Q-Step™1, Yorkshire Bioscience Ltd., UK). The following two primers described by White et al. (1990) were used to amplify the 18S rRNA: NS1, (5`GTAGTCATATGCTTGTCTC3`), and NS2, (5`GGCTGCTGGCACCAGACTTGC3`). Electrophoresis was performed at 80 V with 0.5 x TBE as running buffer in 1.5 % agarose/ 0.5 x TBE gels and then the gel was stained in 0.5 µg/cm^3 (w/v) ethidium bromide solution and destained in deionized water. The DNA sequencing of PCR products was run on an automated DNA sequencer (ABI 3700 capillary sequencer, Macrogene, Korea). The obtained 18S rRNA sequences were submitted to GenBank to acquire accession numbers.

Table 1. Different isolates of *R. solani* used in the present study.

Isolate no.	Host plant		Anastomosis group	Source
	Latin name	Common name		
YEG1	*Phaseolus vulgaris* L.	Common bean	AG-2-2 IIIB	Al-Dakahlia
YEG2	*Phaseolus vulgaris* L.	Common bean	AG-2-2 IIIB	Al-Dakahlia
YEG3	*Phaseolus vulgaris* L.	Common bean	AG-2-2 IIIB	Kafr Al-Sheikh
YEG4	*Phaseolus vulgaris* L.	Common bean	AG-2-2 IIIB	Al-Dakahlia
YEG5	*Vicia faba* L.	Broad bean	AG-2-2 IIIB	Al-Gharbia
YEG6	*Phaseolus vulgaris* L.	Common bean	AG-4 HG-I	Al-Sharkia
YEG7	*Capsicum annuum* L.	Bell pepper	AG-4 HG-I	Al-Giza
YEG8	*Lycopersicon lycopersicum* L.	Tomato	AG-4 HG-I	Damitta
YEG9	*Cucumis sativus* L.	Cucumber	AG-2-2 IIIB	Al-Dakahlia

Sequence analysis

The obtained 18S rRNA sequences were analyzed using the basic local alignment search tool (BLAST) at NCBI database (http://blast.ncbi.nlm.nih.gov) (Altschul et al., 1997).

Pathogenicity test

Pathogenicity test of the isolated fungi was carried out on four different cultivars of common bean plant (Giza 3, Giza 6, Contendor and Rajma) to determine the pathogenic potentialities (virulence) of the different isolates of (*R. solani*), which were isolated from different districts on different common bean cultivars. The most aggressive isolate and the most susceptible cultivar were used for further investigations. Pots (20 cm in diameter) were sterilized by immersing them in 5% formaline solution for 15 min and left for one week until complete formaline evaporation. Pots were filled with disinfested soil at the rate of 2.5 kg/pot; clay: sand (2:1, v/v). Inocula of the tested fungi were prepared by growing each fungus isolate in bottles containing sterilized sorghum grain medium and incubated at 25 ± 2°C for 15 days. Soil infestation was achieved by mixing the inoculum of each fungus with the upper layer of the soil at the rate of 2% (w/w) potential inoculum. The infested soil was mixed thoroughly and irrigated every two days for a week before planting to stimulate the fungal growth and ensure its distribution in the soil. Five healthy common bean seeds were sown in each pot. Three pots were used as replicates for each isolate and plant cultivar, and three un-infested pots were used as control (Mathew et al., 2012). The common bean seeds used in this experiment were obtained from the Central Administration for Seed Certification, Ministry of Agriculture, Egypt. Plants were irrigated when necessary. All pots were kept in a glass house under natural conditions. The disease severity (DS) was determined by recording the percentage of un-emerged seeds (seed rot and pre-emergence) 15 days after sowing as well as percentage of dead plants (post-emergence) 45 days after sowing. The percentage of healthy survival plants were also recorded 60 days after sowing (Carling et al., 1999).

RESULTS

Isolation, purification and identification of the pathogen

Naturally diseased plants of different types (common bean, broad bean, bell pepper, tomato and cucumber) exhibiting typical symptoms of root rot disease were collected. Out of these surveys, nine isolates of the pathogen were obtained and purified using the hyphal tip technique. These isolates were identified as *R. solani* (Table 1).

Anastomosis grouping

Nine isolates of *R. solani* were assigned to AG according to hyphal anastomosis with tester isolates from AG 1 to AG 10. Of these, six isolates belonged to AG 2-2 IIIB, while the other three isolates belonged to AG 4 HG-I (Table 1). Considerable variation in the color of the fungal culture, zonation and sclerotial formation and distribution on PDA plate was observed among the nine isolates after a 14-day growth at room temperature in a continuous darkness. All isolates that paired with the AG 2-2 IIIB tester isolate were characterized by light to dark-brown color, which had definite concentric rings with few sclerotia. While, the three isolates that paired with AG 4 HG-I tester isolates were characterized by the absence of zonation and scleotia.

Molecular identification using 18S-rRNA gene

Genetic DNA for the nine isolates was subjected to PCR to amplify the 18S-rRNA gene. The amplified PCR amplicones were then subjected to DNA sequencing. The results showed that all the isolates were *R. solani* with sequence identity 99% which revealed that these isolates comes from one ancestor, but the analysis based on rRNA-18S sequences failed to group them into different distinct groups on the bases of AGs. The obtained rRNA-18S sequences were submitted to GenBank under accession numbers as shown in Table 2.

Pathogenicity test

All obtained isolates of *R. solani* were subjected to

Table 2. Accession numbers for the rRNA-18S sequences submitted to GenBank.

Isolate no.	Length (bp)	Accession number
YEG1	530	FJ588592
YEG2	598	FJ588591
YEG3	628	FJ588598
YEG4	521	FJ588597
YEG5	597	FJ588590
YEG6	668	FJ588596
YEG7	594	FJ588589
YEG8	532	FJ588594
YEG9	528	FJ588593

Table 3. Pathogenicity of different isolates of *R. solani* on common bean plants.

Isolate no.	Damping -off			Root rot		Damping -off			Root rot	
	Seed rot	Pre-emergence	Post-emergence	No.	Severity*	Seed rot	Pre-emergence	Post-emergence	No.	Severity
			Giza 3					Giza 6		
Control	0	0	0	0	0	0	0	0	0	0
YEG1	7	4	1	3	2.67	3	10	1	1	3
YEG2	15	0	0	0	0	15	0	0	0	0
YEG3	12	2	1	0	0	12	2	1	0	0
YEG4	15	0	0	0	0	15	0	0	0	0
YEG5	14	0	1	0	0	11	2	1	1	3
YEG6	5	4	3	3	1	0	4	2	9	2.56
YEG7	15	0	0	0	0	15	0	0	0	0
YEG8	14	0	1	0	0	13	2	0	0	0
YEG9	6	4	3	2	2.5	3	3	3	6	1.83
Total mortality (%)			94.07			Total mortality (%)		87.41		
			Contendor					Rajma		
Control	0	0	0	0	0	0	0	0	0	0
YEG1	2	11	1	1	3	4	6	2	3	3
YEG2	7	8	0	0	0	8	7	0	0	0
YEG3	0	9	2	4	2.5	0	9	3	3	2.67
YEG4	10	5	0	0	0	15	0	0	0	0
YEG5	0	5	3	7	1.57	4	8	2	1	3
YEG6	1	0	1	13	2.38	1	3	0	11	1.5

Table 3. Contd.

YEG7	5	5	2	3	2.67	4	9	2	0	0
YEG8	1	8	4	2	2.5	5	6	2	2	1
YEG9	0	1	3	11	2.73	3	2	3	7	2.43
Total mortality (%)			69.63			Total mortality (%)		80		

* Damage assessed on a scale ranging from 0 to 4, in which 0 = no damage and 4 = all shoots and roots dead (Carling et al., 1999).

pathogenicity test to determine the most aggressive one.

The results illustrated in Table 3 indicated that all isolates have the potency to cause seed rot, pre-emergence, post-emergence damping-off and root rot diseases, where they caused mortality ranging from 13.33 to 100%. But *R. solani* isolated from Al-Dakahlia (YEG4) recorded the highest value of mortality percentage (100%) with all of the tested bean cultivars.

In order to determine the most susceptible bean cultivar to Rhizoctonia root rot disease, four bean cultivars were tested (Giza 3, Giza 6, Contendor and Rajma).

All tested bean cultivars manifested the disease symptoms (Table 3), but Giza 3 was the most susceptible one. It showed the highest value of total mortality (94.07%).

DISCUSSION

Variability in disease symptoms, host range, and geographical location of *R. solani* isolates suggests that there are several strains of *R. solani* (Meinhardt et al., 2002). Of nine tested isolates, six belonged to AG 2-2 IIIB while the other three isolates belonged to AG 4 HG-I (Figure 1). These findings agree with the results of Bohlooli et al. (2005), who identified AGs of *R. solani* from root rotted bean plants as AG4, AG4HGII and AG2-2-2B.

Generally, symptoms where observed on common bean plants infected with *R. solani* include damping-off, roots and hypocotyl rots, web blight and aerial blight (Meinhardt et al., 2002; Godoy-Lutz et al.; 2008; Mikhail et al, 2010). But, damping-off, roots and hypocotyl rots are mainly associated with the *R. solani* anastomosis group AG-2-2 IIIB or AG-4 (Hagedorn, 2005; Mikhail et al, 2010).

Of 290 isolates of *R. solani* isolated from diseased dry bean and soybean roots and/or hypocotyls and foliage, Muyolo et al. (1993) found that all root/ hypocotyl isolates were AG-2-2 IIIB or AG-4, while, foliar isolates belonged to AG-1-IB. At the same time, all AG-2-2 IIIB or AG-4 tested isolates failed to cause foliar symptoms. This ensured the specificity of the two AGs (AG-2-2 IIIB or AG-4) with the root and/or hypocotyl rots symptoms.

BLAST analysis for the rRNA-18S sequences of the nine isolates at NCBI database showed that all the isolates were *R. solani* with sequence identity 99% with that in the database which revealed that these isolates comes from one ancestor, but it failed to group them into different distinct groups on the bases of AGs. In conclusion, these results showed that the analysis based on the rRNA-18S sequences is not enough to distinguish between them on the bases of AGs. So, in the future studies, we suggest further analysis using rRNA-ITS region sequences in order to differentiate between them according to AGs (Sharon et al., 2007).

Results of pathogenicity test showed that, all isolates were virulent and caused seed rot, pre-emergence, post-emergence damping-off and root rot diseases in varied extents. At the same time, all the tested bean cultivars manifested the diseases symptoms. These results are in agreement with that of Mahmoud et al. (2007), who investigated the pathogenicity of different isolates of *R. solani* isolated from root/hypocotyle rotted plants of various types (cotton, clover and broad bean) on common bean plant and found that all isolates were pathogenic and caused seed rot, pre-emergence, post-emergence damping-off and root rot diseases. The broad host range makes *R. solani* an economically important plant pathogen.

Susceptibility to *R. solani* may be due to polygalacturonase, which may partially degrade pectate (Balali and Kowsari, 2004). *R. solani* generally attacks seedlings at the ground level (hypocotyls) and grows downwards into the roots. Meristimatic tissues of seedlings are susceptible to *R. solani*.

As tissues mature, they become increasingly resistant to *R. solani* due to the conversion of pectin to calcium pectate, which is unaffected by the polygalacturonase produced by the fungus or due to an increase in the cuticle thickness that leads to decrease in exudation and consequent infection cushion formation (Stockwell and Hanchey, 1982).

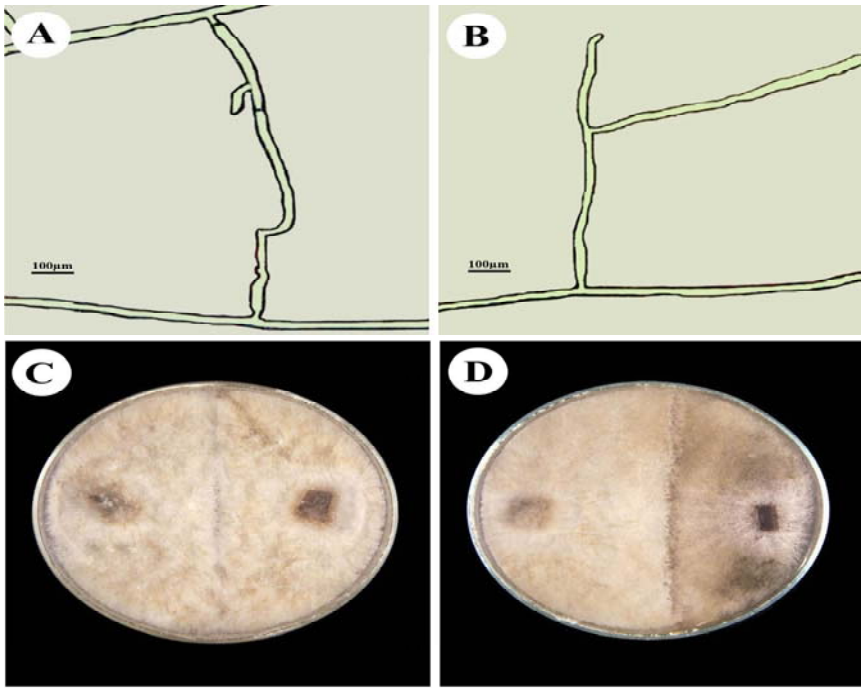

Figure 1. Microscopic hyphal fusion (anastomosis) between two isolates belonging to the same AG (A and B). Macroscopic compatibility between two isolates belonging to the same AG (C), macroscopic incompatibility between two isolates belonging to two different AGs (D).

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Research Center of Teachers College, King Saud University for funding this work. Our deep gratitude is extended to Dr. Khalid Ghoneem (Plant Pathology Research Institute, Agricultural Research Center) for his sincere help in the fungal identification. Hearty thanks go to Prof. Dr. Shiro Kuninaga (Health Sciences University of Hokkaido, Japan) for providing tester isolates of *R. solani*.

REFERENCES

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997). Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

Balali GR, Kowsari M (2004). Pectic zymogram variation and pathogenicity of *Rhizoctonia solani* AG-4 bean (*Phaseolus vulgaris*) isolates in Isfahan, Iran. Mycopathology 158:377-384.

Bohlooli A, Okhovvat SM, Javan-Nikkhah M (2005). Identification of anastomosis group of *Rhizotonia solani*, the causal agent of seed root and damping-off of bean in Iran. Commun. Agric. App. Biol. Sci. 70:137-141.

Carling DE (1996). Grouping in *Rhizoctonia solani* by hyphal anastomosis interaction. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (eds.), *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 35-46.

Carling DE (2000). Anastomosis groups and subsets of anastomosis groups of *Rhizoctonia solani*. In: Proceedings of the 3rd International Symposium on *Rhizoctonia*, Taichung, Taiwan, p.14 (Abstract).

Carling DE, Baird RE, Gitaitis RD, Brainard KA, Kuninaga S (2002). Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology. 92: 893-899.

Carling DE, Pope EJ, Brainard KA, Carter DA (1999) Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. Phytopathol 89:942-946

Ceresini PC, Shew HD, James TY, Vilgalys R, Cubeta MA (2007). Phylogeography of the Solanaceae-infecting Basidiomycota fungus *Rhizoctonia solani* AG-3 based on sequence analysis of two nuclear DNA loci. BMC Evol. Biol. 7:163.

Edwards K, Johnstone C, Thompson C (1991). A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. Nucleic Acids Res. 19:1349.

Fenille RC, de Souza NL, Kuramae EE (2002). Characterization of *Rhizoctonia solani* associated with soybean in Brazil. Eur. J. Plant Pathol. 108:783-792.

Godoy-Lutz G, Kuninaga S, Steadman JR, Powers K (2008). Phylogenetic analysis of *Rhizoctonia solani* subgroups associated with web blight symptoms on common bean based on ITS-5.8S rDNA. J. Gen. Plant Pathol. 74:32-40.

Gonzalez D, Cubeta MA, Vilgalys R (2006). Phylogenetic utility of indels within ribosomal DNA and β-tubulin sequences from fungi in the *Rhizoctonia solani* species complex. Mol. Phylogenet. Evol. 40: 459–470.

Guillemaut C, Edel-hermann V, Camporota P, Alabouvette C, Richard-molard M, Steinberg C (2003). Typing of anastomosis groups of Rhizoctonia solani by restriction analysis or ribosomal DNA. Can. J. Microbiol. 49:556–568.

Hagedorn DJ (2005). *Rhizoctonia* Root Rot. In: Schwartz HF, Steadman JR, Hall R, Forster RL (eds.). Compendium of Bean Diseases, (2nd Ed.). American Phytopathologycal Society, APS Press, St. Paul, Minnesota, USA. pp. 19-20.

Karaca GH, Ozkoc I, Erper I (2002). Determination of the Anastomosis

Grouping and Virulence of *Rhizoctonia solani* Kuhn Isolates Associated with Bean Plants Grown in Samsun/Turkey. Pak. J. Biol. Sci. 5:434-437.

Kronland WC, Stanghellini ME (1988). Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathol. 78:820-822.

Mahmoud YA, Gaafar RM, Mubarak HM (2007). Genetic diversity among Nile Delta isolates of *Rhizoctonia solani Kü*hn based on pathogenicity, compatibility, isozyme analysis and total protein pattern. Turk. J. Bot. 31:19-29.

Mathew FM, Lamppa RS, Chittem K, Chang XW, Botschnei M, Kinzer K, Goswami, RS, Markell SG (2012). Characterization and Pathogenicity of *Rhizoctonia solani* Isolates Affecting *Pisum sativum* in North Dakota. Plant Dis. 96(5):666 – 672.

Meinhardt LW, Wulff NA, Bellato CM, Tsai SM (2002). Genetic analyses of *Rhizoctonia solani* isolates from *Phaseolus vulgaris* grown in the Atlantic Rainforest Region of São Paulo, Brazil. Fitopatol. Brasil. 27: 259-267.

Mikhail MS, Sabet KK, Omar MR, Asran AA, Kasem KK (2010). Current Rhizoctonia solani anastomosis groups in Egypt and their pathogenic relation to cotton seedlings. Afr. J. Microbiol. Res. 4(5):386-395.

Muyolo NG, Lipps PE, Schmitthenner AF (1993). Anastomosis grouping and variation in virulence among isolates of *Rhizoctonia solani* associated with dry bean and soybean in Ohio and Zaire. Phytopathology 83:438-444.

Priyatmojo A, Escopalao VE, Tangonan NG, Pascual CB, Suga H, Kageyama K, Hyakumachi M (2001). Characterization of a new subgroup of *Rhizoctonia solani* anastomosis group 1 (AG-1-ID), causal agent of a necrotic leaf spot on coffee. Phytopathology 91:1054-1061.

Sharon M, Freeman S, Kuninaga S, Sneh B (2007). Genetic diversity, anastomosis groups and virulence of *Rhizoctonia* spp. isolates from Strawberry. Eur. J. Plant Pathol. 117:247-265.

Stockwell V, Hanchey P (1982). Cytohistochemical techniques for calcium localization and their application to diseased plants. Plant Physiol. 70:244-251.

Sneh B, Burpee L, Ogoshi A (1991). Identification of *Rhizoctonia* species, pp. 133. American Phytopathological Society Press, Saint Paul, USA

Sneh B, Jabaji-Hare S, Neate S, Dijst G (1996). *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Tu CC, Hsieh TF, Chang YC (1996). Vegetable diseases incited by *Rhizoctonia* spp. In: Sneh B., Jabaji-Hare S., Neate S, Dijst G (eds.) *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 369-377.

White TJ, Bruns T, Lee S, Taylor J (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.), PCR protocols: a guide to methods and applications. Academic Press, Inc., New York, USA.