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Occurrence and characterisation of *Rhizoctonia* species causing diseases of ornamental plants in Italy

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Abstract During surveys conducted in 2010–2012 *Rhizoctonia* symptoms were observed on 30 ornamental species in different nurseries located in eastern Sicily (Southern Italy). Eighty-eight isolates of *Rhizoctonia* spp. were obtained from symptomatic leaves, roots and stems. Fifty-six of the isolates were binucleate and 32 were multinucleate *Rhizoctonia*. Characterisation of anastomosis groups (AGs) was performed using morphological characteristics and sequence analysis of the internal transcribed spacer of ribosomal DNA (rDNA-ITS) region. Most isolates collected were *Rhizoctonia solani* AG-4 HG-I (35.2% of all isolates) and one isolate was AG-2-2 IIIB. The binucleate isolates belonged to AG-R (27.3%), AG-A (21.6%), AG-G (12.5%), AG-V (1.1%) and AG-Fb (1.1%). The pathogenicity of 38 representative isolates collected from each host was tested on seedlings or cuttings grown in a growth chamber. All *R. solani* AG-4 HG-I isolates, most of the binucleate AG-R, AG-A and AG-G and AG-V were pathogenic and reproduced symptoms identical to that observed in nurseries, while binucleate AG-Fb and *R. solani* AG-2-2 IIIB isolates were nonpathogenic. This is the first report of the occurrence of *Rhizoctonia* species on some ornamental plants and the first report of binucleate *Rhizoctonia* AG-R and AG-V in Europe.

Keywords Ornamental nurseries · Pathogenicity · Root and foliar symptoms · rDNA sequence homology

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Introduction

Rhizoctonia species are soilborne pathogens causing root and foliar diseases on a wide range of agronomic crops, turfgrasses, ornamental plants, fruit and forest trees worldwide (Sneh et al. 1991; Couch 1995).

Rhizoctonia spp. are classified into three groups based on the differences in nuclei number per cell (Ogoshi 1975; Ogoshi 1987; Ogoshi 1996): multinucleate *Rhizoctonia* (MNR) (teleomorphs: *Thanatephorus* and *Waitea*), binucleate *Rhizoctonia* (BNR) (teleomorphs: *Ceratobasidium* and *Tulasnella*) and uninucleate *Rhizoctonia* (UNR) (teleomorph: *Ceratobasidium*). Each group is composed by different anastomosis groups (AGs). Moreover, several AGs of *R. solani* and binucleate *Rhizoctonia* are subdivided into subgroups that differ for biochemical, genetic and pathogenic characteristics (Sneh et al. 1991, 1998; Priyatmojo et al. 2001; Naito 2004; Aoyagi et al. 1998; Sharon et al. 2006).

Binucleate *Rhizoctonia* spp. have been divided into 21 AGs designated AG-A to AG-U (Ogoshi et al. 1983;

Sneh et al. 1991; Hyakumachi et al. 2005). Of the BNR AGs, AG-J and AG-N are excluded, the representative isolates of AG-M are lost, and AG-T and AG-U reported as new BNR AGs by Hyakumachi et al. (2005) were subsequently confirmed to belong to AG-A and AG-P, respectively (Sharon et al. 2008). Moreover, AG-V and AG-W were recently reported in China (Yang 2013; Yang et al. 2015). BNR were weakly virulent, not-pathogenic or even mycorrhizal or biocontrol agents (Harris et al. 1994; Andersen and Rasmussen 1996; Sneh 1998; Hwang and Benson 2002). However, several studies reported BNR as pathogenic on economically important agricultural and horticultural crops (Priyatmojo et al. 2001; Kuramae et al. 2007; Aiello et al. 2012; Muzhinji et al. 2015; Yang et al. 2015).

The multinucleate species are represented by *R. solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk, *Waitea circinata* var. *oryzae* (anamorph = *Rhizoctonia oryzae* Ryker & Gooch) and *W. circinata* var. *zeae* (anamorph = *Rhizoctonia zeae* Voorhees) (Leiner and Carling 1994) and *W. circinata* var. *circinata* (Toda et al. 2005). *R. solani* is composed of 14 anastomosis groups: AG-1 to AG-10, AG-BI (Sneh et al. 1991), AG-11 (Carling et al. 1994), AG-12 (Carling et al. 1999), and AG-13 (Carling et al. 2002) and three subgroups HG-I, HG-II (Kuninaga and Yokosawa 1984) and HG-III (Stevens Johnk and Jones 2001). *R. solani* is the most widespread species, with a host range that includes over 500 plant species (Farr et al. 1995). Hyphal anastomosis was not used because it does not always provide accurate results and currently, rDNA internal transcribed spacer region (ITS) sequence analysis is the most accurate method to determine AGs and subgroups of *Rhizoctonia* spp. and establish phylogenetic relationships among these (Hyakumachi et al. 2005; Sharon et al. 2008).

Rhizoctonia diseases of ornamental plants can occur and in some cases may cause severe epidemics. A wide range of disease symptoms have been recorded, including root and stem rot, leaf spot, seedlings damping-off and foliar web blight on economic important ornamental species such as *Azalea* spp., *Begonia* spp., *Petunia* × *hybrida*, *Rosa* spp., *Vinca minor* and *Pittosporum tobira* worldwide (Chase 1991; Benson and Cartwright 1996; Hyakumachi et al. 2005; Rinehart et al. 2007). In South Italy, binucleate *Rhizoctonia* and *R. solani* are widespread in nurseries and cause extensive damage to young ornamental plants (Aiello et al. 2008a, b, 2009a, b; Polizzi et al. 2009a, b, c, 2010a, b, c, 2011a, b). Prevention is the first strategy to control Rhizoctonia diseases and in particular the adoption of control measures to reduce primary inoculum (use of healthy plants and sterilized pots and potting media, and the removal of infected plants). Chemical management is the approach most often used to control Rhizoctonia diseases. A wide range of chemicals has been reported as effective and selective against *R. solani* and binucleate *Rhizoctonia*-like fungi (Jager et al. 1991; Haralson et al. 2013). However, different *Rhizoctonia* species and AGs possess a different sensitivity towards fungicides (Kataria et al. 1991; Ueyama et al. 1990; Kataria and Gisi 1996; Benson and Cartwright 1996; Csinos and Stephenson 1999; Virgen-Calleros et al. 2000). Therefore, an accurate diagnosis through the determination of *Rhizoctonia* AGs present in a particular area or in an ornamental nursery is important for the selection of effective disease management strategies and for understanding the distribution and spread of pathogen.

Considering the importance of Rhizoctonia diseases and high economic losses caused by these fungi, surveys were conducted over a 3-year period, in commercial ornamental nurseries located in Catania province, eastern Sicily, Italy. The aims of the present study were to identify the AGs and subgroups of *Rhizoctonia* spp. obtained from ornamentals using morphological characteristics and ITS sequence; and evaluate the pathogenicity of representative *Rhizoctonia* isolates on the ornamental hosts from which they were isolated.

Materials and methods

Field surveys, sample collection and fungal isolation

Surveys were conducted during 2010–2012 in 10 nurseries located in eastern Sicily. The disease incidence was recorded for each host species based on the number of symptomatic plants on the total of those producted. Approximately, 20 plants per species per nursery showing Rhizoctonia-like symptoms were randomly collected for analysis. Small sections (0.2–0.5 cm long) from the edge of symptomatic tissues were surface disinfected with 1.5% sodium hypochlorite for 1 min, rinsed once in sterile distilled water (SDW), dried on sterile absorbent paper and placed on potato dextrose agar (PDA, Oxoid) plates amended with 100 ppm streptomycin sulphate (Sigma-Aldrich). Plates were incubated at 25 ± 1 °C under continuous dark conditions.

Following 48 to 72 h of incubation, hyphae from the margin of colonies with features characteristic of *Rhizoctonia* spp. were placed on PDA plates. After 5 days, single-hyphal or tip were selected and transferred into PDA plates for monomycelic cultures.

A total of 88 isolates were obtained and used for morphological and molecular characterisation. Stock cultureswere stored in tubes on PDA covered with mineral oil.

Morphological characteristics and nuclear conditions

The *Rhizoctonia* isolates were identified morphologically by examining the hyphal branching after 3–4 days of growth on PDA. To distinguish binucleate *Rhizoctonia* isolates from multinucleate *R. solani* isolates, the number of nuclei per hyphal cell was determined. Agar disks (5 mm in diameter) containing mycelium from 2- to 3-day-old cultures growing on 2% water agar (WA, Oxoid) were placed on sterile, glass slides in a moist chamber at 25 °C for 2 to 3 days in the dark. Nuclei were stained with one drop of each 1% safranin O and 3% KOH solution (Bandoni 1979). From each isolate, number of nuclei of 20 cells in hyphae at merging two solutions was determined microscopically at × 400 magnification using an Olympus BX61 microscope.

Molecular characterisation

Genomic DNA from each of the 88 isolates was extracted using a conventional method (Izumitsu et al. 2012). Briefly, small piece of mycelia of each isolate was added to 100 μL TE buffer in a 1.5-mL tube. After the sequential treatments by microwaving twice for 1 min, cooling for 10 min, and centrifuging for 5 min, the supernatant was used as the template DNA for PCR. PCR amplification of the rDNA, including regions of ITS1, 5.8S rDNA and ITS2 for each isolate was performed with the primer set of ITS1-F and ITS4-B (Gardes and Bruns 1993). Amplification was performed in 10 μL reaction mixture containing 2 μL template DNA, 1 μL PCR buffer, 0.5 μL dNTP (2.5 mM), 0.15 μL of each primer (20 μM) and 0.05 μL (5 units/μL) *Taq* DNA polymerase (TaKaRa Bio Inc. Japan). Amplification was performed with a thermal cycler (Verity, Life Technologies Applied Biosystems, USA) with the following program: an initial denaturation at 96 °C for 2.5 min; 40 cycles consisting of denaturation at 96 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s; a final extension at 72 °C for 5.5 min. A 3 μL aliquot of PCR product was separated by electrophoresis on 1.5% (*w*/*v*) agarose gel, stained with ethidium bromide and visualized by UV transilluminator. A 5 μL aliquot of remaining each PCR product was cleaned up for sequencing by the addition of 2 μL ExoSAP-IT (Affymetrix, USA) according to the manufacturer's instructions. One μL of template solution was sequenced using BigDye terminator cycle sequencing kit v. 3.1 (Life Technologies Applied Biosystems, USA) with the same primers used for the PCR amplification. Electrophoretic separation of sequencing products was performed on the Genetic Analyzer 3130xl (Life Technologies Applied Biosystems, USA). The nucleotide sequences generated by the sequencing of each isolate in both directions by primer ITS1-F and ITS4-B were edited and assembled using the combination of Sequencher 5.0.1 (Gene Codes Corp., USA) and Indelligent (Dmitriev and Rakitov 2008) with manual adjustment. Sequences from all isolates were compared with those in the GenBank nucleotide database provided by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) using BLAST algorithm to determine sequence identity and find the closest match based on maximal percent identity. Sequences derived in this study were lodged at GenBank.

Phylogenetic analysis

Sequences of 37 isolates of *Rhizoctonia* spp., selected as representative isolates from different hosts, were used for phylogenetic analysis to confirm the AGs and subgroups recovered. Separate phylogenetic trees were constructed for BNR isolates and *R. solani* isolates. Additional reference sequences of 11 isolates from known AGs worldwide were retrieved from GenBank database and included together with the binucleate *Rhizoctonia* isolates obtained in this work. For *R. solani* tree, 15 reference sequences from the same database were added to our isolates (Table 1). The nucleotide sequences generated by the sequencing were aligned using Clustal W algorithm in MEGA v. 6 (Tamura et al. 2013) and the alignment was corrected manually where necessary. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap (BP) consensus tree inferred from 1000 replicates was considered to represent the evolutionary history of the taxa analyzed (Felsenstein

Table 1 Multinucleate and binucleate *Rhizoctonia* isolates derived from Genebank included in the phylogenetic analysis

Group/ Subgroup	Genbank accession number	Host plant	Origin	Isolate	Reference
AG 1-IA	KF907712	*Brassica oleracea*	Vietnam	HNDD01–3	Nadarajah et al. 2014
AG 3	KF234144	Potato Tubers	South Africa	Rh 6b	Nadarajah et al. 2014
AG 2–2 IIIB	AF354116	Mat rush	Japan	15Rs	Gonzalez et al. 2001
AG 5	HQ629874	*Pisum sativum*	North Dakota	AG5 ND2	Nadarajah et al. 2014
AG 6-HGI	AY154306		Malaysia		Nadarajah et al. 2014
AG 7	AF354099	Soil	Japan	63Rs	Nadarajah et al. 2014
AG 8	DQ355142	Barley	United Kingdom	R28	Nadarajah et al. 2014
AG 9	AF354108	Potato	USA	111Rs	Nadarajah et al. 2014
AG 11	AF354114	Soybean	USA	Roth16	Nadarajah et al. 2014
AG 12	AF153804	*Pterostylis acuminata*	Australia	H1	Pope and Carter 2001
AG 4-HGII	JX843820	Potato	China	GS-25	Yang and Wu 2013
AG 4-HGI	JF699278	Cotton	Northern China	CR-89	Unpublished
AG 4-HGI	JF699280	Cotton	Northern China	CR-254	Unpublished
AG 4-HGI	JQ219361	Potato	China	PT73	Unpublished
AG 2–3	AB054871	Glycine max	Japan	H17–4	Carling et al. 2002
AG 2–2 IIIB	GU811666	*Beta vulgaris*	USA	F517	Strausbaugh et al. 2011
AG-L	AB196653	Soil	Japan	FKO-2-26	Hua et al. 2014
AG-K	FJ492158	*Beta vulgaris*	USA	F523	Fang et al. 2013
AG-A	AY927315	Strawberry	Italy	R2	Fang et al. 2013
AG-A	AF354092	Soil	Japan	C-662	Fang et al. 2013
AG-G	AY927325	Strawberry	Italy	R18	Fang et al. 2013
AG-G	DQ097889		Japan	C-653	Unpublished
AG-V	HM623624	Ginger	China	DL-YT-06-4-9	Yang 2013
AG-Fb	AB219145		Japan	FKO-2-28	Hua et al. 2014
AG-Fb	FR734294	*Nicotiana tabacum*	Turkey	T-7	Unpublished
AG-R	DQ885781	Ginger	China	J-04-7	Unpublished
AG-R	JX514382	Orchidaceae	Taiwan	Ano_formo3	Unpublished

1985). The evolutionary distances were computed using the Kimura 2-parameter method (Kimura 1980) and are in the units of the number of base substitutions per site. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The analysis involved 34 nucleotide sequences of BNR and 30 nucleotide sequences of *R. solani*. All positions containing gaps and missing data were eliminated (complete deletion option). The tree with BNR was rooted with an isolate of *R. orizae* (accession number: KC590575) as out-group. The tree with *R. solani* was rooted with an isolate of *Athelia rolfsii* (accession number: AY684917).

Pathogenicity

Pathogenicity tests were performed with 38 representative isolates on potted, healthy, seedlings or cuttings of all symptomatic species recovered (Table 2, except *Carissa macrocarpa*) grown in a growth chamber for 4 months. Each experiment was conducted twice and similar results were obtained in both tests. For each experiment three replicates per isolate were used with 20–50 plants per replicate. All plants were inoculated at the base of each stem plants with two 6 mm in diameter mycelial plugs obtained from cultures grown on PDA plates for 5 days at 25 ± 1 °C in the dark. Uninoculated plants served as a control, for

Table 2 Hosts, symptoms and incidence (%) of Rhizoctonia diseases in ornamental nurseries in Sicily (Southern Italy)

Host	Geographical location	Collection year	Symptoms[a]	Incidence %[b]
Carissa macrocarpa (Eckl.) A. DC.	Giarre, Nursery 1	2011	CRR-SR	10
Carissa grandiflora (E. Mey.) A. DC.	Giarre, Nursery 1	2011	CRR-SR	10
Cistus salvifolius L.	Giarre, Nursery 1	2011	CRR-SR	30
Catharanthus roseus (L.) G. Don	Giarre, Nursery 2	2012	CRR-SR	10
Butia capitata (Mart.) Becc.	Giarre, Nursery 1	2012	DO	20
Trachycarpus fortunei (Hook.) H. Wendl.	Giarre, Nursery 1	2012	DO	20
Phormium spp. J.R. Forst. and G. Forst.	Mascali, Nursery 1	2011	CRR	10
Citrus aurantium L.	Giarre, Nursery 4	2010	DO-CRR	50
Citrus volkameriana Tan. and Pasq.	Giarre, Nursery 4	2010	DO-CRR	20
Citrus sinensis Osbeck x *Poncirus trifoliata* Raf.	Giarre, Nursery 4	2012	DO-CRR	20
Chamaerops humilis L.	Giarre, Nursery 1	2010	DO	20
Arbutus unedo L.	Giarre, Nursery 1	2010	CRR-DO	10
Dodonaea viscosa (L.) Jacq.	Riposto, Nursery 1	2010	CRR	15
Eugenia myrtifolia Sims	Giarre, Nursery 3	2010	CRR	10
Phillyrea angustifolia L.	Giarre, Nursery 3	2010	CRR	10
Grevillea sp. R. Br. ex Knight	Riposto, Nursery 2	2010	CRR	10
Lagunaria patersonii (Andrews) G. Don	Giarre, Nursery 1	2010	DO	20
Osteospermum spp. L.	Giarre, Nursery 1	2010	DO	30
Laurus nobilis L.	Giarre, Nursery 1	2010	DO-CRR	10
Murraya paniculata L.	Giarre, Nursery 1	2010	CRR	15
Quercus ilex L.	Giarre, Nursery 6	2010	CRR	10
Thevetia peruviana (Pers.) K. Schum.	Giarre, Nursery 6	2010	CRR	20
Thryptomene saxicola (A. Cunn. ex Hook.) Schauer	Riposto, Nursery 2	2010	CRR	30
Viburnum tinus L.	Mascali, Nursery 2	2010	CRR-SR	20
Passiflora mollissima (Kunth) L.H. Bailey	Giarre, Nursery 5	2011	CRR	20
Tabebuia impetiginosa (Mart. ex DC.) Standl.	Giarre, Nursery 5	2011	DO-CRR	10
Pittosporum tobira (Thunb.) W.T. Aiton	Giarre, Nursery 1	2012	CRR	10
Streptosolen jamesonii (Benth.) Miers	Giarre, Nursery 1	2011	CRR-SR	50
Bignonia sp. L.	Giarre, Nursery 5	2011	CRR	10
Polygala myrtifolia L.	Giarre, Nursery 1	2012	WB-CRR	50

[a] CRR crown and root rot; SR stem rot; DO damping-off; WB web blight

[b] number of symptomatic plants on the total of those producted

all the hosts. After inoculation plants were covered with a plastic bag for 48 h and maintained at 25 ± 1 °C and 95% relative humidity (RH) under a 12-h fluorescent light/dark regimen. All plants were irrigated 2–3 times per week and examined weekly for disease symptoms. Disease incidence (DI) was assigned to each host species and isolate by determining the percentage of seedlings or cuttings with symptoms of Rhizoctonia disease after 7 days to 4 months from pathogen inoculation.

Results

Field surveys, sample collection and fungal isolation

Symptoms referable to *Rhizoctonia* spp. were detected over a 3-year period in 10 nurseries investigated and on 30 different ornamental species (Figs. 1 and 2). Rhizoctonia diseases were observed in the period from March to November during propagation stage on unrooted and rooted

Fig. 1 Field symptoms referable to *Rhizoctonia* spp. detected on rooted cuttings and seedlings. **a-b** *Cistus salvifolius*; **c** *Polygala myrtifolia*; **d** *Streptosolen jamesonii*; **e** *Tabebuia impetiginosa*; **f** *Trachycarpus fortunei*; **g** *Chamaerops humilis*

cuttings (1–4 months old) in greenhouse and on established plants (1–4 years old) in open field. Diseases incidence varied, approximately, from 10 to 50%, according to the host species (Table 2). The symptoms observed consisted of crown and root rot, stem rot, damping-off and web blight (Table 2). Early in the disease development, crown and stem rot was characterized by water-soaked lesions at the soil line that turned light reddish brown to dark brown and expanded to girdle the stem and internal brown discolouration of cortical tissues; root rot sometimes occurs in association with these symptoms. The infected roots become dark brown or black and were partially or completely destroyed. As a consequence of root and stem rot, basal leaves initially turned chlorotic and gradually became necrotic and sometimes infected plants wilted and died. Damping-off

Fig. 2 Field symptoms referable to *Rhizoctonia* spp. detected on potted plants and seedlings. **a**-**b** *Dodonaea viscosa*; **c** *Viburnum tinus*;**d** *Catharanthus roseus*; **e** *Thryptomene saxicola*; **f-g** *Carissa grandiflora*

consisted of the decay of the stem at soil level, causing it to fall over because it has not yet thickened supporting tissue. Symptoms of either web blight or aerial blight included interveinal and marginal irregular necrotic lesions that progress to total leaf necrosis and leaf-drop. During nursery production, especially under the hot humid conditions (22–30 °C and 85–95% RH), the web-like brown mycelium of the pathogen covered portions of the infected plants and resulted in brown patch disease. The web blight symptom was associated with crown and root rot. Unrooted cuttings and cuttings during rooting stage were susceptible to infection.

Morphological characteristics and nuclear conditions

All isolates showed typical features of *Rhizoctonia* spp. including branching at right angles with constriction at the base of hyphae and septum near the point of origin. Fifty-six of the isolates recovered were binucleate, the other 32 isolates were multinucleate.

Molecular characterisation and phylogenetic analysis

Analysis of the rDNA-ITS region using the BLAST algorithm (against Genbank database) revealed that 31 isolates belonged to *R. solani* AG-4-HGI, 1 to AG-2-2 IIIB, 24 to binucleate AG-R, 19 to AG-A, 11 to AG-G, AG-Fb and AG-V. The similarity range of 79 isolates was from 98% to 100% while nine isolates showed an sequence identity lower (from 85% to 97%) (Table 3). Highest sequence similarities (98–100%) were observed among our *R. solani* AG-4, AG-2-2 IIIB and binucleate *Rhizoctonia* AG-Fb, AG-V and AG-G isolates and representative isolates from Genbank. Binucleate AG-R and AG-A isolates showed an sequence similarity with isolates from Genbank lower (from 93% to 99% and 85% to 99%, respectively).

The optimal tree for *R. solani* with the sum of branch length = 0.56051825 was shown in Fig. 3. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test were shown next to the branches (BP analysis; Fig. 3). There were a total of 342 positions in the final dataset. *R. solani* AG-4 HG-I and AG-2-2 IIIB isolates clustered with representative isolates from Genbank with BP values of 95% and 98%, respectively (Fig. 3).

The optimal tree for BNR with the sum of branch length = 0.49361651 was shown in Fig. 4. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test were shown next to the branches (BP analysis; Fig. 4). There were a total of 308 positions in the final dataset. All BNR isolates formed clades together with representative isolates with bootstrap supports of 98% (AG-A and AG-Fb), 97% (AG-R), 96% (AG-V) and 94% (AG-G) (Fig. 4).

Pathogenicity

Of thirty-eight isolates, thirty *Rhizoctonia* isolates tested were pathogenic to the different original hosts inoculated and produced symptoms identical or similar to those observed on diseases plants in the nurseries (Table 3). Eight isolates were not pathogenic. The DI (%) caused by *Rhizoctonia* species on different hosts ranging from 75 to 100% after 7 days to 4 months (Table 3).

All *R. solani* AG-4 HG-I isolates were pathogenic and caused 100% of DI on *Trachycarpus fortunei*, citrus species, *Chamaerops humilis*, *Osteospermum* sp., *Tabebuia impetiginosa* and *Streptosolen jamesonii*, *Arbutus unedo*, *Lagunaria patersonii*, and *Bignonia* sp., whereas lower DI on *Murraya paniculata* and *Thevetia peruviana* (80% and 75%, respectively).

Binucleate AG-A isolates caused high DI (from 80% to 100%) on *Dodonaea viscosa*, *Cistus salvifolius*, *Quercus ilex*, *Thryptomene saxicola*, *Passiflora mollissima*, *Carissa grandiflora*, and *Catharanthus roseus* but were non pathogenic on *Viburnum tinus* and *Grevillea* sp. The binucleate AG-V isolate (61 LNO) was pathogenic to original host (*Laurus nobilis*) and caused 100% of DI. Binucleate AG-R isolates didn't cause disease on *C. grandiflora* but were pathogenic to *Butia capitata, C. salvifolius*, *P. mollissima* and *Polygala myrtifolia* causing DI from 90% to 100%. Binucleate AG-G isolates were pathogenic and caused DI from 85% to 100% on *Pittosporum tobira*, *V. tinus*, *Phillyrea angustifolia*, *Quercus ilex* and *C. roseus* but not were pathogenic to *B. capitata* and *Phormium* spp.

On some host species two AGs were recognized and both were pathogenic (AG-A and AG-R on *C. salvifolius* and *P. mollissima*, AG-A and AG-G on *C. roseus* and *Q. ilex*) whereas on other species only one AG recovered was pathogenic (AG-A on *C. grandiflora*, AG-R on *B. capitata* and AG-G on *V. tinus*).

None AGs recognized from *Phormium* spp. (AG-Fb, AG-2-2 IIIB and AG-G) was pathogenic on this species. The pathogens were re-isolated from the artificially inoculated plants and identified as previously described, completing Koch's postulates. No symptoms were observed on control plants.

Discussion

In this study, 88 *Rhizoctonia* isolates were recovered from 30 ornamental species in eastern Sicily (Southern Italy) over a 3-year period, and their AGs and subgroups determined. Fifty-six isolates were binucleate *Rhizoctonia* and 32 were multinucleate and were identified as *R. solani*. *Rhizoctonia* symptoms were observed in 10 ornamental nurseries and included damping-off, crown, root and stem rot and web blight

Table 3 Identity and pathogenicity of *Rhizoctonia* isolates collected from ornamental plants

Sample Code	Host	Genotypic AG	Identity	Accession number	Pathogenicity	Incidence (%)
01 CMA	*Carissa macrocarpa*	AG-R	450/459 (98%)	KX171277		
02 CMA		AG-R	450/458 (98%)	KX171280		
03 CMA		AG-R	449/458 (98%)	KX171278		
04 CMA		AG-R	449/458 (98%)	KX171281		
05 CMA		AG-R	687/695 (99%)	KX171284		
06 CMA		AG-R	470/482 (98%)	KX171279		
07 CMA		AG-R	431/464 (93%)	KX171283		
08 CGR[a]	*Carissa grandiflora*	AG-R	686/694 (99%)	KX171285	-	
09 CGR[a]		AG-A	478/497 (96%)	KX171328	+	80
10 CGR		AG-R	686/694 (99%)	KX171287		
11 CGR		AG-R	686/694 (99%)	KX171288		
12 CGR		AG-A	671/674 (99%)	KX171302		
13 CGR		AG-A	477/495 (96%)	KX171327		
14 CSA[a]	*Cistus salvifolius* L	AG-A	735/744 (99%)	KX171303	+	100
15 CSA		AG-A	722/725 (99%)	KX171304		
16 CSA[a]		AG-R	333/349 (95%)	KX171329	+	90
17 CSA		AG-R	423/447 (95%)	KX171298		
18 CRO[a]	*Catharanthus roseus*	AG-A	726/733 (99%)	KX171320	+	90
19 CRO		AG-A	728/738 (99%)	KX171322		
20 CRO		AG-A	409/422 (97%)	KX171321		
21 CRO		AG-A	376/424 (89%)	KX171330		
22 CRO		AG-A	560/657 (85%)	KX171331		
23 CRO		AG-G	753/759 (99%)	KX171310		
24 CRO		AG-A	424/432 (98%)	KX171326		
25 CRO		AG-A	726/733 (99%)	KX171323		
26 CRO[a]		AG-G	745/746 (99%)	KX171311	+	90
27 BCA[a]	*Butia capitata*	AG-G	727/745 (98%)	KX171312	-	
28 BCA[a]		AG-R	702/707 (99%)	KX171286	+	100
29 TFO[a]	*Trachycarpus fortunei*	AG-4 HG-I	698/707 (99%)	KX130705	+	100
30 TFO		AG-4 HG-I	728/736 (99%)	KX130695		
31 TFO		AG-4 HG-I	698/709 (98%)	KX130696		
32 TFO		AG-4 HG-I	698/711 (98%)	KX130697		
33 TFO		AG-4 HG-I	725/733 (99%)	KX130706		
34 TFO		AG-4 HG-I	594/602 (99%)	KX130715		
35 PSP[a]	*Phormium* spp.	AG-G	636/668 (95%)	KX171313	-	
36 PSP		AG-G	747/755 (99%)	KX171314		
37 PSP		AG-G	726/729 (99%)	KX171318		
38 PSP[a]		AG-Fb	682/690 (99%)	KX171300	-	
39 PSP[a]		AG-2-2 IIIB	512/512 (100%)	-	-	
40 CAU[a]	*Citrus aurantium*	AG-4 HG-I	528/529 (99%)	KX130711	+	100
41 CAU		AG-4 HG-I	525/525 (100%)	KX130698		
42 CVO[a]	*Citrus volkameriana*	AG-4 HG-I	708/709 (99%)	KX130712	+	100
43 CVO		AG-4 HG-I	505/505 (100%)	KX130699		
44 CVO		AG-4 HG-I	512/512 (100%)	KX130700		
45 CVO		AG-4 HG-I	724/735 (99%)	KX130718		
46 PTR[a]	*Poncirus trifoliata*	AG-4 HG-I	526/527 (99%)	KX130713	+	100

Table 3 (continued)

Sample Code	Host	Genotypic AG	Identity	Accession number	Pathogenicity	Incidence (%)
47 PTR		AG-4 HG-I	528/529 (99%)	KX130714		
48 CHU[a]	*Chamaerops humilis*	AG-4 HG-I	706/707 (99%)	KX130720	+	100
49 CHU		AG-4 HG-I	707/712 (99%)	KX130719		
50 AUN[a]	*Arbutus unedo*	AG-4 HG-I	695/697 (99%)	KX130701	+	100
51 DVI[a]	*Dodonaea viscosa*	AG-A	677/701 (97%)	KX171324	+	100
52 EMY[a]	*Eugenia myrtifolia*	AG-A	725/729 (99%)	KX171305	+	90
53 PAN[a]	*Phillyrea angustifolia*	AG-G	743/745 (99%)	KX171315	+	100
54 GSP[a]	*Grevillea* sp.	AG-A	560/657 (85%)	KX171332	-	
55 LPA[a]	*Lagunaria patersonii*	AG-4 HG-I	514/521 (99%)	KX130717	+	100
56 LPA		AG-4 HG-I	703/707 (99%)	KX130721		
57 LPA		AG-4 HG-I	527/527 (100%)	KX130707		
58 OSP[a]	*Osteospermum* sp.	AG-4 HG-I	519/521 (99%)	KX130722	+	100
59 OSP		AG-4 HG-I	531/531 (100%)	KX130723		
60 OSP		AG-4 HG-I	527/527 (100%)	KX130724		
61 LNO[a]	*Laurus nobilis*	AG-V	560/562 (99%)	KX171301	+	
62 MPA[a]	*Murraya paniculata*	AG-4 HG-I	691/694 (99%)	KX130709	+	80
63 MPA		AG-4 HG-I	527/527 (100%)	KX130710		
64 MPA		AG-4 HG-I	695/702 (99%)	KX130716		
65 QIL[a]	*Quercus ilex*	AG-A	605/611 (99%)	KX171325	+	100
66 QIL[a]		AG-G	581/583 (99%)	KX171306	+	100
67 TPE[a]	*Thevetia peruviana*	AG-4 HG-I	529/529 (100%)	KX130702	+	75
68 TSA[a]	*Thryptomene saxicola*	AG-A	605/606 (99%)	KX171307	+	100
69 VTI[a]	*Viburnum tinus*	AG-G	744/746 (99%)	KX171316	+	100
70 VTI[a]		AG-A	601/613 (98%)	KX171308	-	
71 PMO	*Passiflora mollissima*	AG-R	670/682 (98%)	KX171282		
72 PMO[a]		AG-R	667/682 (98%)	KX171299	+	100
73 PMO[a]		AG-A	609/610 (99%)	KX171309	+	100
74 TIM[a]	*Tabebuia impetiginosa*	AG-4 HG-I	626/632 (99%)	KX130703	+	100
75 TIM		AG-4 HG-I	686/702 (98%)	KX130725		
76 PTO[a]	*Pittosporum tobira*	AG-G	751/754 (99%)	KX171317	+	85
77 PTO		AG-G	750/754 (99%)	KX171319		
78 SJA[a]	*Streptosolen jamesonii*	AG-4 HG-I	695/697 (99%)	KX130708	+	100
79 BSP[a]	*Bignonia* sp.	AG-4 HG-I	696/698 (99%)	KX130704	+	100
80 PMY[a]	*Polygala myrtifolia*	AG-R	637/642 (99%)	KX171289	+	100
81 PMY		AG-R	637/641 (99%)	KX171290		
82 PMY		AG-R	641/646 (99%)	KX171291		
83 PMY		AG-R	615/619 (99%)	KX171292		
84 PMY		AG-R	635/641 (99%)	KX171293		
85 PMY		AG-R	637/641 (99%)	KX171294		
86 PMY		AG-R	639/643 (99%)	KX171295		
87 PMY		AG-R	635/641 (99%)	KX171297		
88 PMY		AG-R	637/645 (99%)	KX171296		

[a] isolates used in the pathogenicity tests; +pathogenic; − nonpathogenic

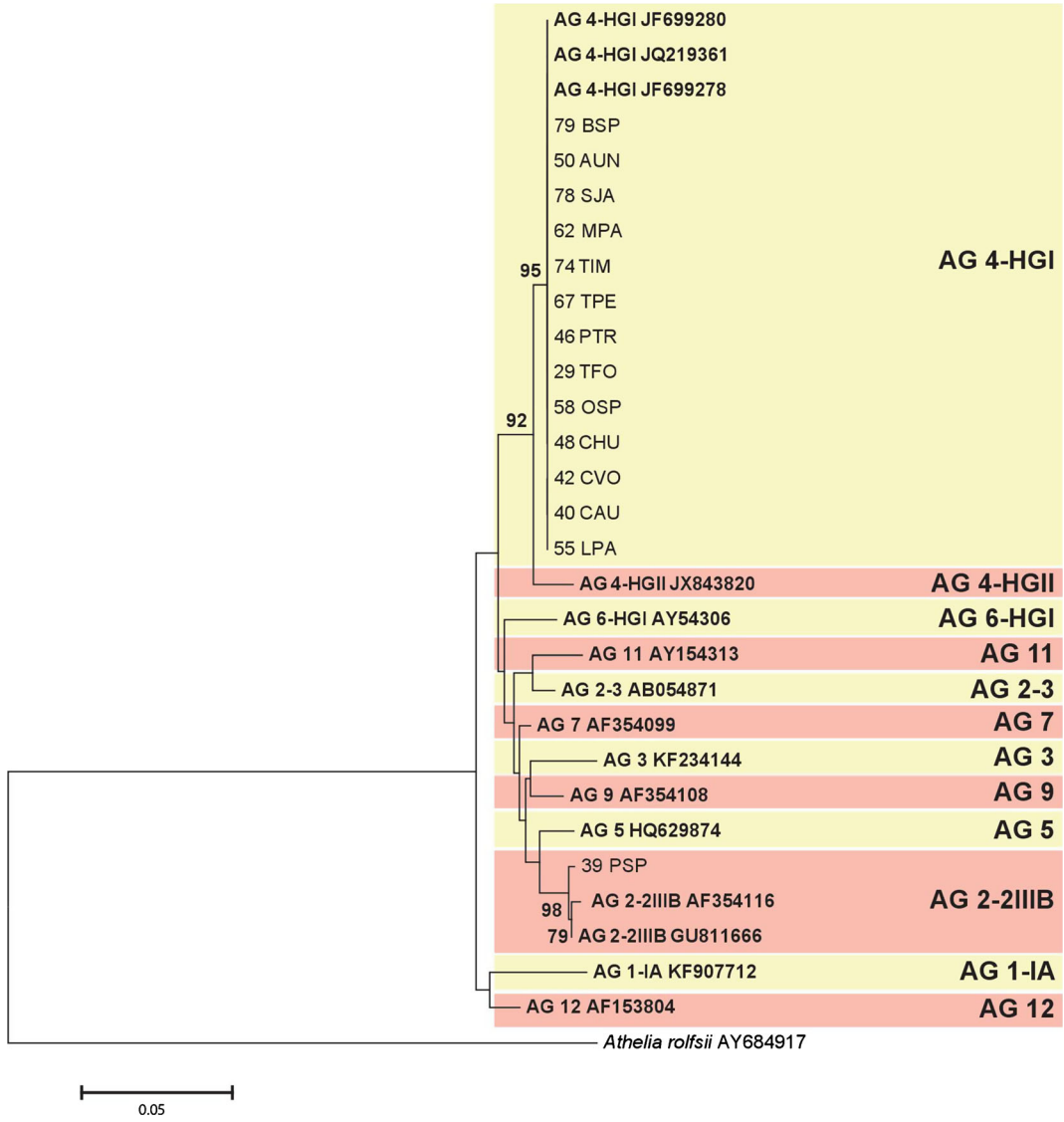

Fig. 3 Neighbor-joning tree derived from alignment of 30 *Rhizoctonia solani* isolates and the outgroup *Athelia rolfsii* (AY684917). The isolates in bold are the representative isolates from Genbank. Bootstraps indicated on the branched nodes are only given for those branches with value higher than 70

thought damping-off was mainly associated with *R. solani* AG-4 while crown and stem rot was caused primarily from BNR. Infections were observed in the period from March to November during young growing stages in greenhouse (i.e., seeding, rooting and pot transplanting) and during summer (June–July) in the containerised field-grown species, probably, favored by optimal conditions for growth of the *Rhizoctonia* species.

Based on results of characterisation and of phylogenetic analysis, the most prevalent anastomosis group was *R. solani* AG-4 subgroup HG-I (35.2%) followed by binucleate *Rhizoctonia* AG-R (27.3%), AG-A (21.6%) and AG-G (12.5%), while only one isolate of AG-2-2 IIIB (*R. solani*), AG-Fb and AG-V was recognized.

In the pathogenicity tests, *R. solani* AG-4 isolates, the majority of binucleate AG-R, AG-A and AG-G isolates and AG-V isolate were pathogenic to original host, fulfilling Koch's postulates. None AGs recognized from *Phormium* spp. (AG-Fb, AG-2-2 IIIB and AG-G) and the AG from *Grevillea* sp. (AG-A) were pathogenic on

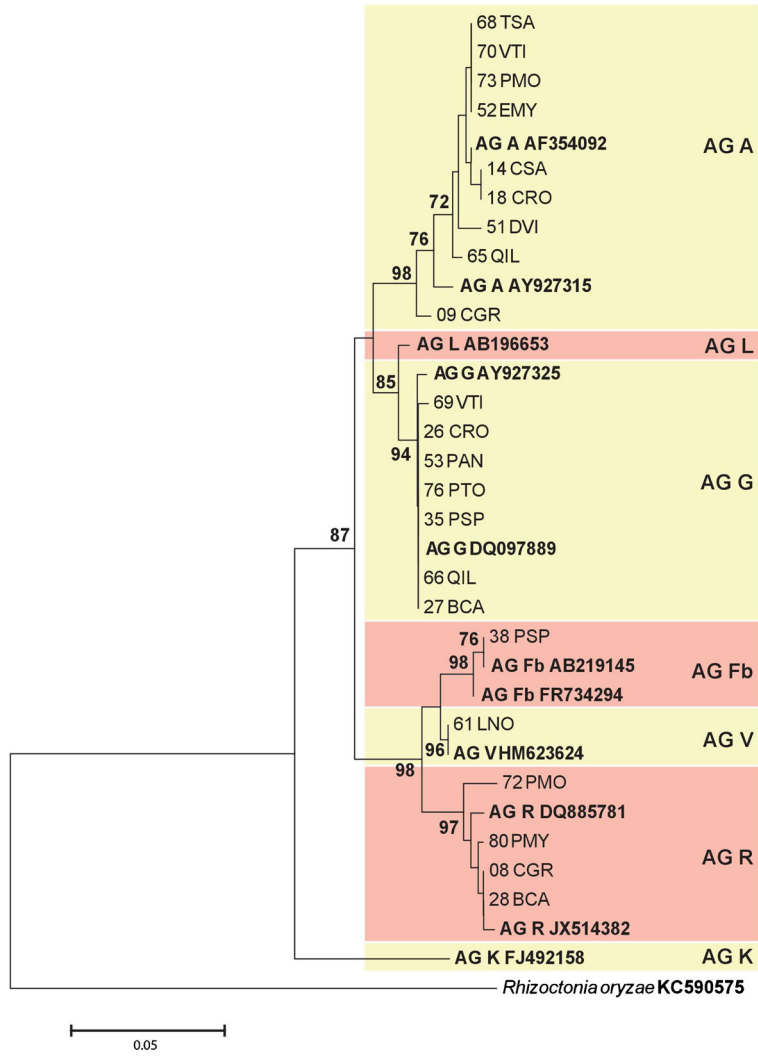

Fig. 4 Neighbor-joning tree derived from alignment of 34 binucleate *Rhizoctonia* isolates and the outgroup *Rhizoctonia orizae* (KC590575). The isolates in bold are the representative isolates from Genbank. Bootstraps indicated on the branched nodes are only given for those branches with value higher than 70

these species that seem not be susceptible to Rhizoctonia disease. Our study revealed a diversity in the composition of *Rhizoctonia* populations recovered from ornamental nurseries (binucleate AG-R, AG-A, AG-G, AG-V and AG-Fb; *R. solani* AG-4 and AG-2-2 IIIB). Moreover, in some cases, different binucleate AGs were recovered from the same host and both were pathogenic (AG-A and AG-R on *C. salvifolius* and *P. mollissima*, AG-A and AG-G on *C. roseus* and *Q. ilex*). In other cases, only one AG among those recovered appeared pathogenic to original host (AG-A on *C. grandiflora*, AG-R on *B. capitata* and AG-G on *V. tinus*. These results are consistent with reports that show that the host range of individual AG differs (Ogoshi 1996). Various studies have documented that certain hosts are susceptible to specific AGs and not others (Ohkura et al. 2009). These results could be also explained with the presence of some non-pathogenic or hypovirulent binucleate *Rhizoctonia* isolates. Several studies reported the presence of these isolates that colonize plant roots and can have an antagonistic activity against pathogenic *Rhizoctonia* isolates (Harris et al. 1994; Hwang and Benson 2002; Khan et al. 2005) or considered as mycorrhizal species (Andersen and Rasmussen 1996).

R. solani is widespread in ornamental nurseries in Italy (Aiello et al. 2008a, b, 2009a, b; Polizzi et al. 2009b, 2010a, c, 2011a, b; Garibaldi et al. 2003, 2006, 2009a, b) where represents a very limiting factor for ornamental plants cultivated in Sicily (Southern Italy). The pathogen has a very broad host range worldwide (Farr et al. 1995) and among these several ornamental species have been reported in the literature (Chase 1991;

Sneh et al. 1991; Priyatmojo et al. 2001). However, no reports have been published of diseases caused by *Rhizoctonia* spp. on *Citrus volkameriana*, citrange, *T. fortunei*, *A. unedo*, *T. peruviana* and *Bignonia* sp.

BNR AG-A and AG-G are the two most common groups associated with root rot on strawberry in the world (Martin 2000) and widespread in strawberry-growing areas in Northern Italy (Manici and Bonora 2007). Recently, have been also reported on ornamentals in Southern Italy (Polizzi et al. 2009a, c, 2010b) but have not been reported diseases caused by *Rhizoctonia* AG-A on *Carissa* spp., *C. roseus*, *Eugenia* sp., *Q. ilex*, and *Rhizoctonia* AG-G on *P. angustifolia*, *P. tobira* and *C. roseus*.

R. solani was reported in Florida on *C. grandiflora*, *Carissa* spp. and *Eugenia* sp. (Alfieri et al. 1972, 1984), and *R. solani* and *Rhizoctonia* sp. were reported on *Quercus* spp. (Collado et al. 1996; Mulenko et al. 2008). Chase (1991) reported a binucleate *Rhizoctonia* sp. causing aerial blight and root rot of *P. tobira* while Alfieri et al. (1984) reported *R. solani* in Florida. Binucleate *Rhizoctonia* sp. and *R. solani* has been reported on *C. roseus* in United States (Alfieri et al. 1984; Chase 1991; Holcomb and Carling 2002) and *R. solani* AG-1 IB in Italy (Garibaldi et al. 2006).

AG-R, the binucleate AG most frequently found in this survey, has only been reported in the USA, Australia, Brasil and China (Burpee et al. 1980; Sumner 1985; Yang et al. 2006, Rinehart et al. 2007), and was not previously reported to be present in Italy (Europe), as well as AG-V recently reported only in China (Yang 2013). This study is the first report of Rhizoctonia disease on *C. salvifolius*, *B. capitata*, *P. myrtifolia* caused by AG-R and on *L. nobilis* caused by AG-V. In addition, this is also the first report of binucleate *Rhizoctonia* AG-R and AG-V in Europe.

The results of these surveys showed that, overall, BNR were the most prevalent *Rhizoctonia* species isolated from ornamental nurseries in Sicily. The higher frequency of binucleate *Rhizoctonia* spp. recovered could be explained with the cropping history of the study site as reported by other authors (Gill et al. 2000, 2001, 2004; Schroeder and Paulitz 2008). However, data from a European survey shows that *Rhizoctonia* spp. can be expected to be present in the soil irrespective of the previous crop in the field (Goll et al. 2014).

The high incidence of disease observed in several ornamental nurseries in the last years and the frequency and distribution of AGs will therefore depend on other factors such as climatic conditions and farming practice (Virgen-Calleros et al. 2000). The environmental conditions during propagation of plants in the greenhouse heated and irrigated overhead could provides ideal condition for Rhizoctonia disease development (warm temperatures and high humidity), such as the use of non-disinfected substrate. The containerised plants production could have a role in promoting infections because the ornamental plants are frequently stressed, remain containerised throughout production, and several wounds could be incurred during transplanting. Moreover, the predominant potting component in field-grown nurseries in most of the eastern Sicily is autochtone volcanic soil mixed with peat and perlite or vermiculite to assure plant stability and proper development. In eastern Sicily, potato-growing area are widespread near to the ornamental nurseries, and different reports show the high susceptibility of potato to binucleate *Rhizoctonia* and *R. solani* (Muzhinji et al. 2015). The use of non-disinfected soil taken from these infected area or the use of soil recycled could represent a possible source of pathogen inoculum and increase infection risks by Rhizoctonia. Prevention, thus, is the first strategy to control Rhizoctonia diseases but an accurate diagnosis through the determination of *Rhizoctonia* AGs present in a particular area or in an ornamental nursery is important for selection of effective disease management strategies.

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