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Characterization and pathogenicity of fungi and oomycetes associated with root diseases of date palms in Oman

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ABSTRACT

Date palm is the most important crop in Oman and the Arabian Peninsula. A study was conducted to investigate fungal and oomycete pathogens associated with root diseases of date palms in Oman. Isolations were done from date palm roots showing root rot/necrosis symptoms. The root samples were collected from 111 date palm trees representing 29 different date palm cultivars. Morphological and molecular identification of the isolated fungi and oomycetes showed that they belong to 34 different fungal and oomycete species. *Fusarium solani* (27%), *Ceratocystis radicicola* (25%) and *Lasiodiplodia theobromae* (19%) were found to be the most common pathogens associated with root diseases of date palms. Pathogenicity tests on seedlings of date palm cv. Khalas showed that 21 fungal and oomycete species are pathogenic on date palm seedlings. The pathogenic species produced root rot, root necrosis or wilt symptoms upon inoculation on date palm seedlings. A total of 1, 7, 13 and 10 fungal and oomycete pathogens were found to be aggressive, moderately aggressive, weak and non-pathogenic on date palm seedlings, respectively, with *C. radicicola* being the most aggressive. Among the 21 pathogenic species, 13 are reported in this study for the first time as new root pathogens of date palm on a global basis. These include *Ceratocystis omanensis*, *Cochliobolus hawaiiensis*, *Exserohilum rostratum*, *Corynascus kuwaitiensis*, *Fusarium brachygibbosum*, *Fusarium acuminatum*, *Fusarium redolens*, *Fusarium thapsinum*, *Nigrospora sphaerica*, *Phoma multirostrata*, *Pythium indigoferae*, *Pythium spinosum* and *Pythium ultimum* var. *ultimum*. In addition, this study reports for the first time the occurrence of 22 fungal and oomycete species in Oman.

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1. Introduction

Date palm is the most important crop in Oman and the Arabian Peninsula. Total world production of dates was estimated at 7.5 million tons in 2009. The Arabian Peninsula produces more than one third of the world's production of dates (FAO, 2011). In Oman, there are about 8 million date palm trees grown all over the country with a total production of 278,000 tons in 2009, making Oman among the top 9 produces of dates in the world (FAO, 2011). In addition, date palms occupy over 80% of the area devoted for fruit production in the country, with over 200 different date palm cultivars. Um Sella, Mabsali, Kisab, Nighal, Fardh, Shahla, Khenizi and Khalas are the most widely grown cultivars, where they make up more than 70% of total date production in Oman.

Date palms are vulnerable to a number of disease problems. *Fusarium oxysporum* (Schlechtendahl) f. sp. *albedinis* (Killian & Maire)-induced bayoud disease is one of the most serious and widely studied diseases of date palm (El-Modafar, 2010; Fernandez and Tantaoui, 1994; Zaid et al., 2002). Despite its restricted distribution to a few countries, the disease has wiped out millions of date palm trees especially in Morocco and Algeria (El-Modafar, 2010; Zaid et al., 2002). *Ceratocystis radicicola* Bliss (anamorph *Thielaviopsis punctulata*) is also an important pathogen of date palm worldwide. It has a wide distribution in the Arabian Peninsula, Africa, Europe and the USA and causes a number of diseases in date palms which includes black scorch, root rot, trunk rot and wilt (Abdullah et al., 2009; Bliss, 1941; Linde and Smit, 1999; Suleman et al., 2001, 2002). Other diseases on date palm include balat disease, false smut, lethal yellowing and some other foliar and root diseases (Alhudaib et al., 2007; Elliott et al., 2004; Namsi et al., 2007; Zaid et al., 2002).

In Oman, wilt is increasingly becoming the major constraint for date palm production (Al-Raisi et al., 2011). Affected date palm trees show weakened growth, followed by yellowing of older leaves and then death of the highly affected trees. In pathogenicity tests, *C. radicicola* was found to cause root rot followed by weakened

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growth, wilt and finally death (Al-Raisi et al., 2011). Several pathogenic fungi, including *Ceratocystis* spp., *Fusarium* spp., *Botryodiplodia* spp., *Phoma* spp., *Phomopsis* spp, have been shown to associate with root diseases of date palm trees in other parts of the world (El-Deeb et al., 2007; Linde and Smit, 1999; Zaid et al., 2002). It is therefore not clear whether *C. radicicola* is the only root pathogen of date palms in Oman. In addition, little is still known about fungal and oomycete pathogens which are associated with date palm roots on a global basis. Lack of knowledge in this area establishes a barrier towards effective management of root pathogens of date palm in Oman and elsewhere.

The main objective of this study is to characterize fungal and oomycete pathogens associated with root diseases of date palm in Oman. Specific objectives include: (1) to investigate fungal and oomycete pathogens associated with root rot/necrosis of date palms in Oman, (2) to investigate pathogenicity of the isolated fungi and oomycetes on date palms. This should help establish solid information about distribution of pathogens affecting date palms in Oman which will be important in delineating future disease management programs for these pathogens.

2. Materials and methods

2.1. Pathogens associated with root rot of date palms

A survey was conducted over 2009 and 2010 in 9 different districts located in 4 different geographical regions in Oman in order to characterize the main pathogens associated with root diseases of date palm (Fig. 1). The survey covered 2–6 farms per district (except 1 farm in BidBid), with 3–18 date palm trees (avg. 12) per farm (Table 1). About 3–10 root pieces which showed necrosis and/or root rot symptoms were collected from each tree from the top 5 cm of the soil level. Root pieces were washed using tap water, surface sterilized using 1% sodium hypochlorite (NaOCl), washed in sterile distilled water (SDW) and then blotted dry on sterile filter paper. Three 5-mm root pieces were placed in each Petri-dish containing 2.5% potato dextrose agar (PDA, Oxoid, Hampshire, England). Two Petri-dishes were used for each sample

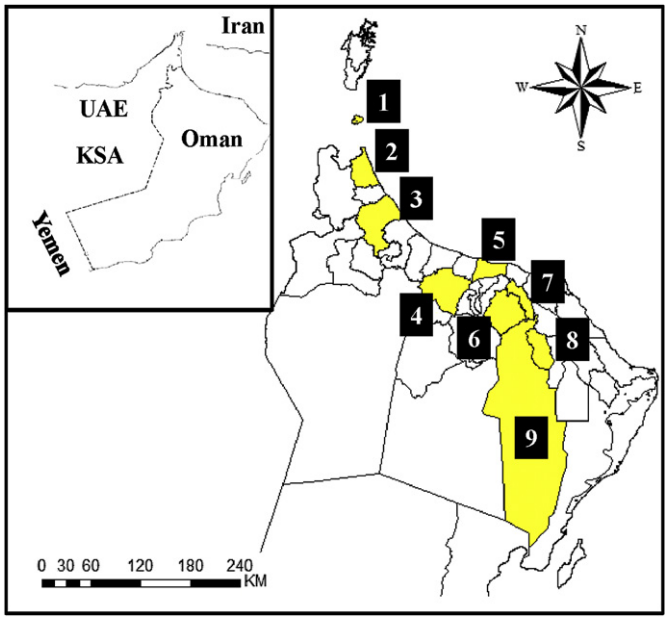

Fig. 1. A map showing the four main date palm growing regions in Oman from which collection of samples was done. The districts are as follows: 1 – Madha, 2 – Shinas, 3 – Sohar, 4 – Rustaq, 5 – Barka, 6 – Samael, 7 – BidBid, 8 – Ibra and 9 – Mudhaibi.

Table 1
Date palm growing regions, districts, farms and cultivars which were included in the survey.

Region	District	Sample size (farms/district)	Sample size (trees/district)	Date palm cultivars
Batinah	Barka	5	15	Khalas, Salani, Nighal, Khenizi, Handhal, Jibri, Lolo, Sha'eeri, Kisab, Fardh, Mabsali, Nashoo, BuJozi, Um Al-Sella, Miznajat Nabir, Boushari, Qash Amer, Masl, Qash Jareza
	Shinas	5	15	
	Rustaq	6	18	
	Sohar	3	15	
Interior	BidBid	1	3	Khalas, Nighal, Handhal, Kisab
	Samael	2	6	
Musandam	Madha	5	15	Nighal, Kisab, Shahla, Saygee, Ain Baqar
Sharqiya	Ibra	5	15	Barni, Khalas, Fardh, Zabad, Kisab, White Hilali, Handhal, Nashoo, Jibri, Madlooki, Rataibi, Mabsali, Nighal, Khenizi
	Mudhaibi	3	9	
Total		35	111	29

and the plates were maintained at room temperature (22 °C ± 2) for 3–7 days. Fungal and oomycete growth coming out of the root pieces was subcultured into PDA plates. This was followed by producing pure cultures using mycelium tip culture (Al-Sa'di et al., 2007). Isolates were preserved at room temperature in PDA slants amended with 10 mg L^{-1} rifampicin and 100 mg L^{-1} ampicillin.

2.2. Molecular characterization of the isolated fungi and oomycetes

Preliminary identification of the isolated fungi and oomycetes was based on morphological characteristics (Barnett and Hunter, 1998; Leslie and Summerell, 2006; Plaats-Niterink, 1981). However, identity of all the isolated fungi and oomycetes was further confirmed using sequences of the internal transcribed spacer region of the ribosomal DNA (ITS rDNA) as described by Al-Sadi et al. (2011b).

Mycelium was harvested from the fungi and oomycetes which were grown on 2.5% PDA for 3–7 days, followed by freeze drying. A method modified from Lee and Taylor (1990) as described by Al-Sa'di et al. (2007) was used for DNA extraction from mycelium. The ITS rDNA region of the fungal and oomycete isolates was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). The PCR reaction mixture consisted of 0.4 μM of each primer, ~25 ng of DNA samples, PuReTaq™ Ready-To-Go™ PCR beads (GE Healthcare) and Milli-Q water up to a final volume of 25 μl. The PCR reaction conditions were as per Al-Sadi et al. (2011b).

Amplification of the ITS region was checked using gel electrophoresis. About 5 μL of each reaction mixture was run on a 1.5% agarose gel in 0.5× Tris–borate–EDTA buffer (TBE) at 100 V for 60 min. PCR products were purified from dNTPs and primers using the UltraClean PCR Clean-up Kit (MO BIO, Carlsbad, CA) and samples were sequenced at Macrogen Inc. (Seoul, Korea) using the same primers used for amplification.

The forward and backward ITS sequences for each isolate were first aligned together and edited using ChromasPro. A representative sequence from each identical set of sequences was compared to worldwide collections of sequences deposited at the National

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