

Molecular characterization and pathogenicity of *Alternaria* species on wheat and date palms in Oman

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Abstract This study was conducted to investigate the *Alternaria* species associated with leaf spot of date palm and wheat in Oman. Out of 98 date palm leaf samples and 146 wheat leaf samples, *Alternaria* was isolated from 27 and 23% of the samples developing leaf spot symptoms, respectively. Identification of *Alternaria* isolates using sequences of the internal transcribed spacer region of the ribosomal RNA (ITS rRNA), glyceraldehyde-3-phosphate dehydrogenase (GPDH), translation elongation factor (TEF) and RNA polymerase II subunit (RPB2) genes, showed that the isolates belong to seven *Alternaria* species or species complexes. *A. burnsii* - *A. tomato* and *A. arborescens* species complexes (58 and 4%, respectively) and *A. alternata* (38%) were the species recovered from the symptomatic date palm leaves. *A. alternata* (67%), *A. burnsii* - *A. tomato* species complex (15%), *A. jacinthicola* (3%), *A. ventricosa* (3%), *A. slovaca* (6%) and *Alternaria caespitosa* (6%) were isolated from wheat. Pathogenicity test showed that tested isolates of *A. alternata* (DPM19, WDK12), *A. burnsii* - *A. tomato* species complex (DPM31), *A. jacinthicola* (WBR4) and

A. slovaca (WDK9, WDK7) were pathogenic on date palm, while *A. alternata* (DPM19, WDK12), *A. burnsii* - *A. tomato* species complex (DPM31, WDK11) and *A. slovaca* (WDK9, WDK7) were pathogenic on wheat. This is the first report of date palm and wheat as new hosts for *A. burnsii* - *A. tomato* species complex and the first reports of *A. burnsii* - *A. tomato* species complex, *A. caespitosa*, *A. slovaca*, and *A. ventricosa* in Oman. The study shows that several species of *Alternaria* are associated with leaf spot in date palm and wheat in Oman, with some isolates having the ability to cause infection in both hosts.

Keywords Date palms · Pathogenicity · Phylogeny · Section *Alternaria* · Section *Infectoriae* · Wheat

Introduction

Species of *Alternaria* are ubiquitous pathogens and saprophytes which occur worldwide in different habitats such as atmosphere, soil and on dead or dying plant tissues. The genus *Alternaria* comprises important pathogens of several vegetable, oil and cereal crops (Villalobos et al. 2017; Gur et al. 2017; Esmaeili Taheri et al. 2017; Moslemi et al. 2017; Abata et al. 2016; Munhuweyi et al. 2016; Thomma 2003). It can cause several diseases including leaf spot, leaf blight, black point and many other diseases. Beside yield losses, species of *Alternaria* are responsible for spoilage of commodities during transportation, storage and processing, which may lead to the reduction of marketing

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value and serious economic losses (Villalobos et al. 2017; Xu et al. 2017; Pane et al. 2016).

Previous studies in Oman showed that *Alternaria alternata* and *Alternaria arborescens* are the most common *Alternaria* species associated with leaf spots of date palms (Al-Sadi et al. 2012) while *Alternaria alternata* is the most common *Alternaria* species associated with leaf spot of wheat (Moghal et al. 1993). Besides causing diseases in plants, *Alternaria* species have been recovered from soil and are also responsible to the decay of dead tissue and leaves (Abed et al. 2013; Al-Riyami et al. 2009).

Date palm is the most important crop in the Sultanate of Oman. It occupies more than 80% of the agricultural area allocated for the fruit crops. Oman is currently the world eighth's largest producer of dates, producing more than 270,000 metric tons (MT) a year (FAO 2015). Omani people utilized date palms in a convenient way that ensured sustainable life during the history and enable them to build a strong and stable civilization in the early age. Date palm is subject to many diseases that affect all parts of the tree; the roots, stem, leaves and fruits. Those diseases vary upon season to season and country to country depending on the presence of the pathogen and environmental conditions. The severity of the diseases varies from low severity leaf spot to high severity wilt diseases which cause significant economic losses (Al-Sadi et al. 2012; Al-Sadi et al. 2015).

Bread wheat is an important food in the world and in the Sultanate of Oman. Diseases are a major cause of yield loss in wheat in the world. The most common diseases of wheat are rust, covered smut or bunt, loose smut and leaf spots (Junk et al. 2016; Fetch et al. 2015; Al-Sadi 2016). Although date palm is grown in Oman in the presence of several crops, including wheat, little is known about the common *Alternaria* species which can cause infection on both hosts.

Molecular techniques have been applied as alternative assays for the detection of microorganisms. Sequence data from ribosomal DNA and protein coding gene regions have been used to infer phylogenetic relationships among different *Alternaria* species and within the populations of the same species (Gannibal et al. 2014; Pavón et al. 2011; Park et al. 2008; Woudenberg et al. 2015; Lawrence et al. 2013). Also, several genes have been sequenced in *Alternaria* and they proved to be efficient in discriminating closely related species. These include the internal transcribed spacer region of the ribosomal RNA (ITS rRNA), glyceraldehyde-3-

phosphate dehydrogenase (GPDH), translation elongation factor (TEF), RNA polymerase II subunit (RPB2) and *Alternaria* major allergen gene (Alt a 1) (Woudenberg et al. 2013; Lawrence et al. 2016).

One objective is to characterize *Alternaria* species associated with leaf spots in date palm and wheat in Oman. A further objective is to study the pathogenicity of some of the isolates on these two plant species.

Studies on *Alternaria* species on date palm and wheat will help understand the alternative hosts of these pathogens in the country.

Materials and methods

Collection of samples

Samples of date palm leaves and wheat leaves were collected from date palms and wheat developing necrotic spots. The spots on date palm leaves were characterized by dark brown spots on leaflets and on the rachis (Fig. 1). A total of 94 leaf samples were collected from 30 date palm trees (2-3 years old) from Muscat, the capital of Oman. Also 146 leaf samples of wheat were collected from Batinah (41), Buraimi (21), Dakiliya (42), Dhahira (27) and Sharqiya (15) from symptomatic leaves developing brown necrotic spots on the leaves, one to two samples from each plant (Fig. 1). The date palm and wheat samples were collected during February to March, 2014.

Isolation

Symptomatic leaves were cut into small pieces (approx. $0.5 \times 0.5 \text{ cm}^2$) and surface sterilized with 1% sodium hypochlorite for one minute. The leaf pieces were washed in sterile distilled water and excess liquid was removed by sterile filter paper. Leaf pieces were transferred to 2.5% potato dextrose agar (PDA) amended with 10 mg^{-1} rifampicin and incubated at 25°C up to 10 days. Fungal growth, which is typical of *Alternaria*, was transferred to new plates and pure cultures were obtained from single spores.

DNA Extraction

Preliminary identification based on morphological characteristics showed that a total of 59 isolates (26 from date palm and 33 from wheat) are typical of *Alternaria*

Fig. 1 Leaf spot symptoms on date palm (A) and wheat (B) leaves



species. DNA was extracted from the 59 *Alternaria* isolates grown on 2.5% potato dextrose agar (PDA) as described by Al-Sadi et al. (2011). Fungal mycelium was scraped from the surface of PDA and freeze dried. The freeze dried mycelium was ground and 600 µl of lysis buffer (50 mM TrisHCl, 50 mM EDTA, 3% SDS, 1% 2-mercaptoethanol) was added and the resulted mixture was incubated at 65 °C for 1 h on water bath. Phenol: chloroform: isoamyl alcohol (25:24:1) was added and the mixture was centrifuged. Then 0.6 volumes of isopropanol and sodium acetate (0.3 M, final concentration) were added to the supernatant. After incubation and centrifugation, the pellet was washed with 70% ethanol and dried. Finally the pellet was

dissolved in 100 µl sterile distilled water and stored at −20 °C until used.

Polymerase chain reaction (PCR) and sequencing

Identification of *Alternaria* to the species level was based on the use of a combination of five different primers (Table 1) with the Alt a region used to identify taxa belonging to the *Alternaria* section *Infectoriae*. PCR for each gene was performed with 25 µl PCR mixture using PuRe-Taq™ Ready-To-Go™ PCR beads (GE Healthcare, Buckinghamshire, UK), 1 µl of each primer (0.4 mM), 1 µl DNA and 22 µl sterile distilled water. The PCR conditions for the ITS region were as described by

Table 1 Primers used for the sequencing of *Alternaria* genes

genes	Primer names	sequences	References
ITS	ITS1	5'TCCTCCGGTTATTGAT ATGC'3	(De Hoog & Gerrits van den Ende 1998)
	ITS4	5' CATCGAGAAGTTCGAGAAGG'3	(White et al. 1990)
TEF1	EF1-728F	5' CAT CGA GAA GTT CGA GAA GG'3	(Carbone & Kohn 1999)
	EF1-986R	5'TAC TTG AAG GAA CCC TTA CC'3	(Carbone & Kohn 1999)
RPB2	RPB2-5F2	5'GGGGWGAYCAGAAGAAGGC'3	(Sung et al. 2007)
	RPB2-7cR	5'CCCATRGCTTGYYTTRCCCAT'3	(Liu et al. 1999)
GPDH	gpd 1	5'CAACGGCTTCGGTCGCATTG'3	(Berbee et al. 1999)
	gpd 2	5'GCCAAGCAGTTGGTTGTGC'3	(Berbee et al. 1999)
Alt a 1	Alt-for	5'ATGCAGTTCACCACCATCGC'3	(Hong et al. 2005)
	Alt-rev	5'ACGAGGGTGAYGTAGGCGTC'3	(Hong et al. 2005)

Al-Sadi et al. (2011). PCR conditions for the TEF1 gene consisted of 40 cycles of 30 s at 94 °C, 30 s at 52 °C / 59 °C and 45 s at 72 °C with the final elongation step being 7 min at 72 °C. The partial RPB2 and Alt a 1 gene were amplified as explained by Woudenberg et al. (2013). The PCR conditions for the GPDH gene were 35 cycles with an initial 4 min at 94 °C for activation and a final 10 min at 72 °C for the extension. Each cycle consisted of 60 s at 94 °C, 55 s at 40 °C and 120 s at 72 °C (Berbee et al. 1999). PCR products were purified and sequenced at Macrogen Inc. (South Korea).

Phylogenetic analysis

Multiple sequence alignments were generated with MEGA v. 5.2.2. Initial results showed that our isolates belong to two sections of *Alternaria*; section *Alternaria* and section *Infectoriae* as designated by Lawrence et al. (2013) and Woudenberg et al. (2015). Therefore, two different datasets were used to estimate two phylogenies: a section *Alternaria* tree based on combined ITS + GPDH + TEF + RPB2 regions and section *Infectoriae* tree based on combined ITS + GPDH + TEF + RPB2 + Alt a 1. A Maximum Likelihood (ML) analysis was performed using raxmlGUI v. 1.3 (Silvestro and Michalak 2012). The optimal ML tree search was conducted with 1000 separate runs, using the default algorithm of the program from a random starting tree for each run. The final tree was selected among suboptimal trees from each run by comparing likelihood scores under the GTR + GAMMA substitution model.

Pathogenicity test

Seven isolates of *Alternaria* spp. (*Alternaria alternata*: DPM19, WDK12; *A. slovaca* (*Chmelia slovaca*): WDK7, WDK9; *A. jacinthicola*: WBR4; and *A. burnsii* - *A. tomato* species complex: DPM31, WDK2) were used in pathogenicity test. Pathogenicity test was done on healthy leaves of 21-day-old wheat plants (var. cooly) and on leaves of three-month old date palm seedlings grown from seeds of Khalas cultivar. The seedlings were grown in 15-cm diameter pots containing potting mix and sandy soil at a ratio of 1:1.

Wheat leaves (five leaves per plant) were inoculated with 5 µl spore suspension (approx. 42 ± 3 spores/µl) obtained from 15-day-old cultures (Al-Sadi 2016), two spots on each leaf. There were two seedlings per pot and five replicate pots per treatment. Control seedlings were

inoculated using sterile distilled water. The inoculated seedlings were covered with transparent polyethylene bags for two days post inoculation. Date palm seedlings were inoculated in the same way and all pots were incubated under laboratory conditions (22 ± 2 °C). The development of symptoms was recorded after 10 days. Re-isolations were conducted from the inoculated leaves as described above. Each experiment was repeated twice. Data were analysed using Tukey's Studentized range test (SAS). (Table 2)

Results

Isolation

Isolation from symptomatic date palm leaves resulted in a number of fungal species, with *Alternaria* isolates being the most common (frequency of isolation = 27%). The other common fungi on date palm leaves were *Cladosporium* (10%), *Acremonium* (9%) and *Diaporthe* (7%). However, isolation from symptomatic wheat leaves showed that *Alternaria* (23%), *Bipolaris* (14%) and *Fusarium* (13%) were the most common. Since our focus was on *Alternaria*, we excluded the remaining isolates from further analysis.

Phylogenetic analysis

A total of 59 *Alternaria* isolates were obtained from symptomatic date palm and wheat leaves. The combined ITS, GPDH, TEF and RPB2 alignment was used to resolve the species in the section *Alternaria* (Fig. 2). The alignment comprised 98 strains (including the outgroup taxon *Alternaria alternantherae* (CBS 124392) and the manually adjusted dataset comprised 1869 characters including gaps. Thirty-two isolates from both hosts clustered with *A. alternata* (Fig. 2), and one isolate from date palm (DPM14) clustered with *A. arborescens*. One isolate from wheat (WBR4) can be recognized as *A. jacinthicola*, 20 isolates from both wheat and date palm clustered with representative strains of the species *A. tomato* and *A. burnsii* and were considered as members of a species complex *A. burnsii*-*A. tomato* until further morphological and sequence data is available.

Species relationships in the section *Infectoriae* are shown in Fig. 3. The combined ITS + GPDH + TEF + RPB2 + Alt a 1 alignment comprises 30 strains

Table 2 GenBank accession numbers of the four genes of *Alternaria* isolates collected during this study

Species	Isolate No	Source	Location	GenBank accession				
				ITS	TEF	RPB2	GPDH	Alt a 1
<i>Alternaria alternata</i>	DPM03	Date palm	Muscat	KY484876	KY513910	KY513841	KY524288	
<i>A. alternata</i>	DPM4	Date palm	Muscat	KY484839	KY513855	KY513807	–	–
<i>A. alternata</i>	DPM11	Date palm	Muscat	KY484892	KY513911	KY513847	KY524289	–
<i>A. alternata</i>	DPM16	Date palm	Muscat	KY484879	KY513897	KY513832	–	–
<i>A. alternata</i>	DPM17	Date palm	Muscat	KY484897	MG967622	MG967640	MG967629	–
<i>A. alternata</i>	DPM19	Date palm	Muscat	KY484899	KY513912	KY513842	–	–
<i>A. alternata</i>	DPM20	Date palm	Muscat	KY484900	KY513856	KY513802	KY524254	–
<i>A. alternata</i>	DPM27	Date palm	Muscat	KY484858	KY513913	KY513844	KY524282	–
<i>A. alternata</i>	DPM28	Date palm	Muscat	KY484888	MG967623	MG967641	MG967630	–
<i>A. alternata</i>	DPM34	Date palm	Muscat	KY484878	KY513904	KY513833	–	–
<i>A. alternata</i>	DPM36	Date palm	Muscat	KY484895	KY513881	KY513803	KY524255	–
<i>A. alternata</i>	SOK3	Soil	Muscat	KY484861	KY513885	KY513810	–	–
<i>A. alternata</i>	SOK4	Soil	Muscat	KY484894	MG967624	MG967642	MG967631	–
<i>A. alternata</i>	SOK5	Soil	Muscat	KY484883	KY513892	KY513821	KY524268	–
<i>A. alternata</i>	SOK8	Soil	Muscat	KY484874	MG967625	MG967643	MG967632	–
<i>A. alternata</i>	WB12	Wheat	Barka	KY484875	KY513907	KY513838	–	–
<i>A. alternata</i>	WB15	Wheat	Barka	KY484891	KY513909	KY513840	KY524287	–
<i>A. alternata</i>	WB17	Wheat	Barka	KY484885	MG967626	MG967644	MG967633	–
<i>A. alternata</i>	WB2	Wheat	Barka	KY484877	KY513882	–	KY524258	–
<i>A. alternata</i>	WB8	Wheat	Barka	KY484898	KY513906	KY513843	–	–
<i>A. alternata</i>	WB9	Wheat	Barka	KY484869	KY513894	KY513846	KY524277	–
<i>A. alternata</i>	WBR5	Wheat	Braimi	KY484850	KY513899	–	MG967638	–
<i>A. alternata</i>	WBR7	Wheat	Braimi	KY484870	KY513900	–	MG967639	–
<i>A. alternata</i>	WDH2	Wheat	Dahirah	KY484863	KY513878	KY513804	–	–
<i>A. alternata</i>	WDH3	Wheat	Dahirah	KY484873	KY513903	KY513835	–	–
<i>A. alternata</i>	WDH5	Wheat	Dahirah	KY484864	KY513880	KY513805	KY524256	–
<i>A. alternata</i>	WDK1	Wheat	Dakhilyah	KY484893	KY513905	KY513836	KY524284	–
<i>A. alternata</i>	WDK12	Wheat	Dakhilyah	KY484896	KY513890	KY513824	–	–
<i>A. alternata</i>	WDK13	Wheat	Dakhilyah	KY484867	KY513891	KY513825	KY524272	–
<i>A. alternata</i>	WDK14	Wheat	Dakhilyah	KY484890	KY513895	KY513831	KY524280	–
<i>A. alternata</i>	WDK15	Wheat	Dakhilyah	KY484871	KY513901	KY513845	KY524281	–
<i>A. alternata</i>	WDK3	Wheat	Dakhilyah	KY484865	KY513887	KY513813	KY524269	–
<i>A. alternata</i>	WDK6	Wheat	Dakhilyah	KY484872	KY513902	KY513834	–	–
<i>A. alternata</i>	WS1	Wheat	Sharqyah	KY484889	KY513893	KY513828	KY524275	–
<i>A. alternata</i>	WS2	Wheat	Sharqyah	KY484901	KY513883	KY513811	KY524260	–
<i>A. alternata</i>	WS3	Wheat	Sharqyah	KY484866	KY513888	KY513822	KY524270	–
<i>A. arborescens</i>	DPM14	Date palm	Muscat	KY484887	KY513915	KY513818	KY524265	–
<i>A. caespitosa</i>	WB16	Wheat	Barka	KY484902	MG256667	KY513806	MG256675	–
								MG256668
<i>A. caespitosa</i>	WB6	Wheat	Barka	KY484906	KY513916	–	MG256674	MG256669
<i>A. jacinthicola</i>	WBR4	Wheat	Braimi	KY484856	KY513872	KY513829	–	–
<i>Alternaria</i> sp.	DPM06	Date palm	Muscat	KY484846	KY513862	KY513817	KY524264	–

Table 2 (continued)

Species	Isolate No	Source	Location	GenBank accession				
				ITS	TEF	RPB2	GPDH	Alt a 1
<i>Alternaria</i> sp.	DPM15	Date palm	Muscat	KY484849	KY513864	KY513827	KY524274	–
<i>Alternaria</i> sp.	DPM18	Date palm	Muscat	KY484854	KY513869	KY513851	KY524283	–
<i>Alternaria</i> sp.	DPM21	Date palm	Muscat	KY484841	KY513857	KY513853	–	–
<i>Alternaria</i> sp.	DPM29	Date palm	Muscat	KY484880	KY513858	KY513809	KY524259	–
<i>Alternaria</i> sp.	DPM31	Date palm	Muscat	KY484868	KY513865	KY513820	KY524267	–
<i>Alternaria</i> sp.	DPM35	Date palm	Muscat	KY484859	KY513870	KY513837	KY524285	–
<i>Alternaria</i> sp.	SOK09	Soil	Muscat	KY484855	MG967627	MG967645	MG967634	–
<i>Alternaria</i> sp.	WBR2	Wheat	Braimi	KY484844	KY513860	KY513815	KY524262	–
<i>Alternaria</i> sp.	WBR6	Wheat	Braimi	KY484860	KY513866	KY513830	KY524279	–
<i>Alternaria</i> sp.	WDK11	Wheat	Dakhilyah	KY484845	KY513861	KY513816	KY524263	–
<i>Alternaria</i> sp.	DPM04	Date palm	Muscat	KY484839	KY513855	KY513807	–	–
<i>Alternaria</i> sp.	DPM22	Date palm	Muscat	KY484847	KY513875	–	MG967637	–
<i>Alternaria</i> sp.	DPM23	Date palm	Muscat	KY484881	–	KY513819	KY524266	–
<i>Alternaria</i> sp.	DPM26	Date palm	Muscat	KY484857	–	KY513850	MG967636	–
<i>Alternaria</i> sp.	DPM32	Date palm	Muscat	KY484884	KY513876	KY513848	KY524276	–
<i>Alternaria</i> sp.	SOK6	Soil	Muscat	KY484882	MG967628	MG967646	MG967635	–
<i>Alternaria</i> sp.	WB3	Wheat	Barka	KY484842	KY513873	KY513813	–	–
<i>Alternaria</i> sp.	DPM07	Date palm	Muscat	KY484848	KY513863	KY513826	KY524273	–
<i>Alternaria</i> sp.	DPM10	Date palm	Muscat	KY484853	KY513868	KY513852	–	–
<i>Alternaria</i> sp.	DPM12	Date palm	Muscat	KY484840	KY513871	KY513808	KY524257	–
<i>Alternaria</i> sp.	WDK2	Wheat	Dakhilyah	KY484843	KY513859	–	KY524261	–
<i>A. ventricosa</i>	WDK4	Wheat	Dakhilyah	KY484904	KY513918	KY513854	KY524278	MG256670
<i>Chmelia slovacae</i>	WDK7	Wheat	Dakhilyah	KY484905	KY513919	KY513839	KY524286	MG256671
<i>C. slovacae</i>	WDK9	Wheat	Dakhilyah	KY484903	KY513917	KY513812	MG256673	MG256672

(including strains from wheat and *Alternaria molesta* CBS 548.81 as the outgroup taxon) and 2625 characters including gaps. Two isolates obtained in this study from wheat clustered with *Alternaria caespitosa* (WB6, WB16), one isolate from wheat (WDK4) clustered with *A. ventricular*, and two from wheat (WDK7, WDK9) clustered with *A. slovacae* (Fig. 3).

Alternaria species associated with date palm and wheat

Results indicated the association of three *Alternaria* species with leaf spot of date palm. Isolates belonging to *A. burnsii* - *A. tomato* species complex (58%) and *A. alternata* (38%) were the most common. *A. arborescens* was only recovered at a low frequency (4%; Table 3).

Six *Alternaria* species were isolated from wheat leaves showing leaf spot symptoms. *A. alternata* was the dominant species, recovered from 67% of the symptomatic leaves. It was detected in the five surveyed regions in this study which are Batinah, Buraimi, Dakiliya, Dhahira and Sharqiya. Isolates belonging to *A. burnsii* - *A. tomato* species complex, *A. jacinthicola*, *A. ventricosa*, *A. slovacae* and *A. caespitosa* were recovered at lower frequencies (Table 3).

Fig. 2 Maximum Likelihood tree obtained from the combined ITS, GPDH, TEF and RPB2 sequence alignment analysis of the species in section *Alternaria*. Bootstrap support values above 50% are shown at the nodes and newly generated sequences are in red. *Alternaria alternantherae* (CBS 124392) is used as the outgroup taxon

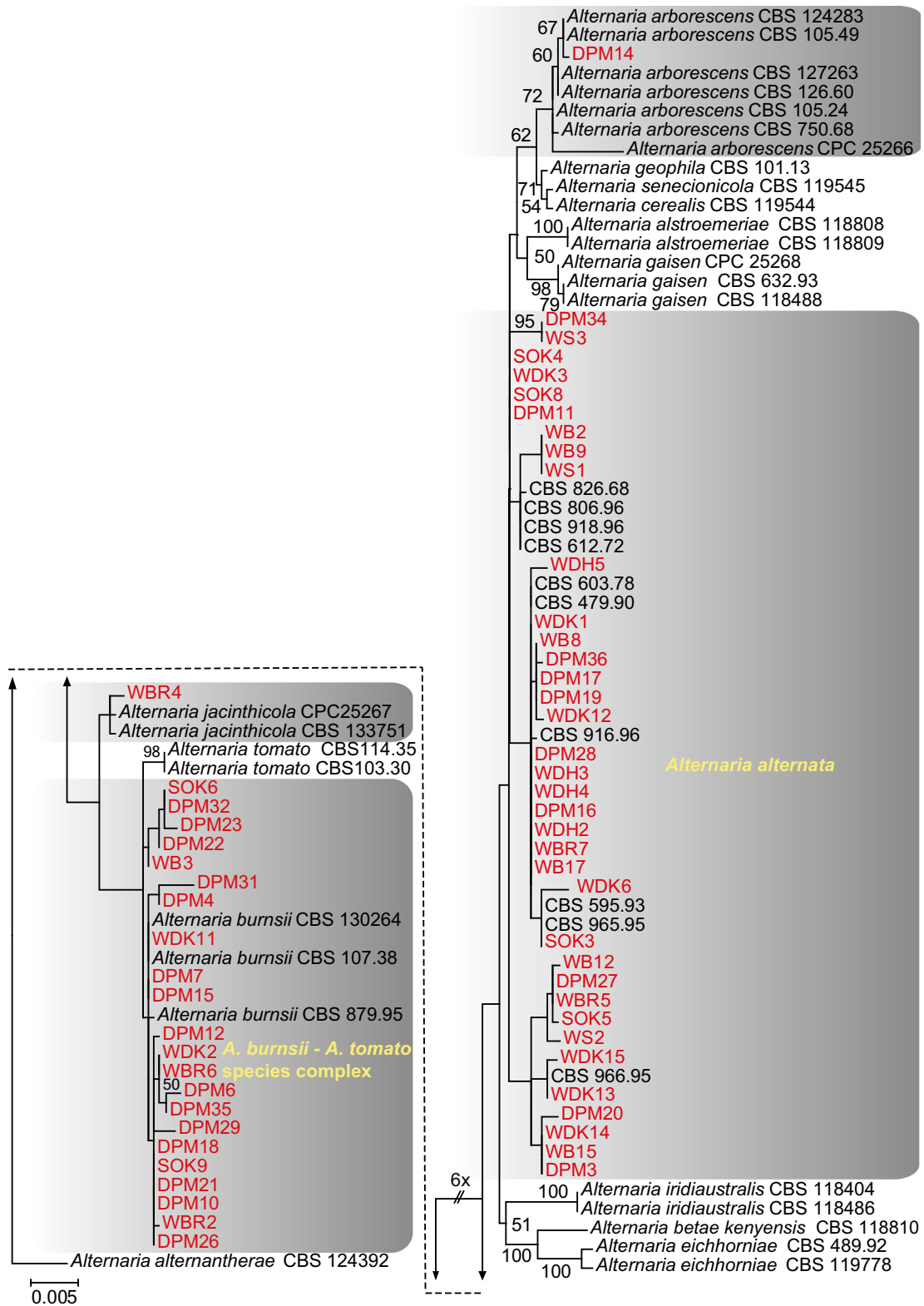
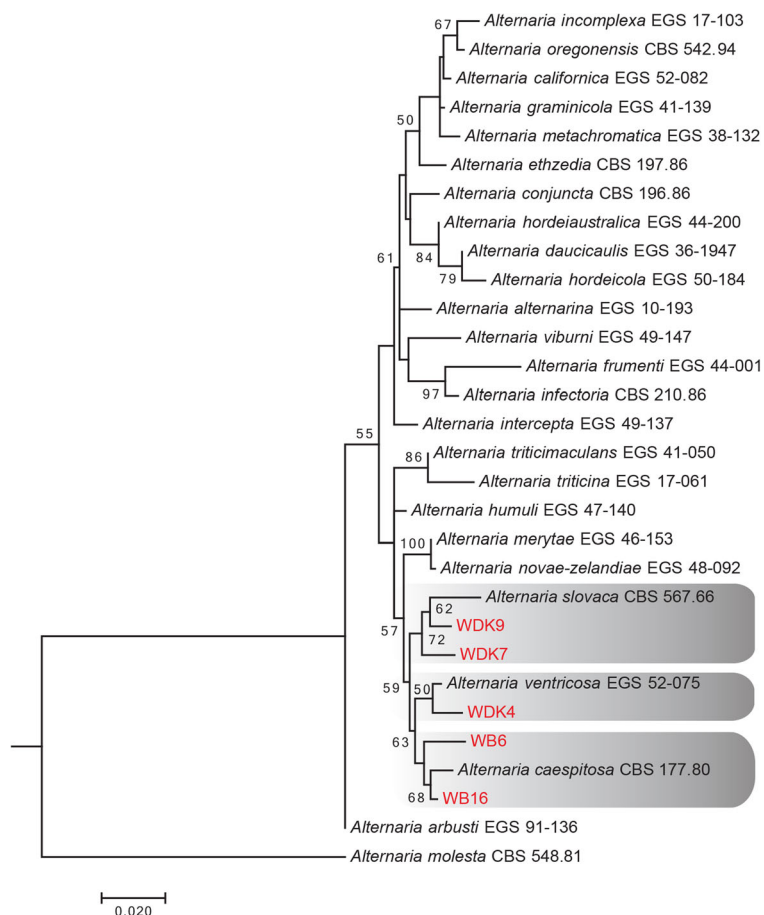


Fig. 3 Maximum Likelihood tree obtained from the combined ITS, GPDH, RPB2, TEF and Alt a 1 sequence alignment analysis of the species in section *Infectoriae*. Bootstrap support values above 50% are shown at the nodes and newly generated sequences are in red. *Alternaria molesta* (CBS 548.81) is used as the outgroup taxon



Pathogenicity test

Inoculation of date palm leaves with different species of *Alternaria* resulted in the production of leaf spot symptoms by most isolates. Isolates of *A. alternata*, *A. slovacica*, *A. jacinthicola* and *A. burnsii* - *A. tomato*

species complex produced necrotic spots ranging in size from 6 to 12 mm in length by 3 - 5 mm in width. The centre of the spots was dark brown to black in color while the borders were light brown, with no differences between species in the shape of color of spots. The isolates showed significant differences in their

Table 3 Relative association (%) of seven *Alternaria* species with leaf spot in date palm and wheat

Species	Date palm	wheat	Distribution of <i>Alternaria</i> from wheat
<i>Alternaria alternata</i>	38	67	Batinah, Buraimi, Dakiliya, Dhahira, Sharqiya
<i>A. Arborescens</i>	4	—	—
<i>A. burnsii</i> - <i>A. tomato</i> complex	58	15	Buraimi, Dhahira
<i>A. jacinthicola</i>	—	3	Buraimi
<i>A. ventricosa</i>	—	3	Dhahira
<i>A. slovacica</i> , (<i>Chmelia slovacica</i>)	—	6	Dhahira
<i>Alternaria caespitosa</i>	—	6	Batinah, Dhahira

(—) indicates that no isolate was recovered

The sample sizes for *Alternaria* isolates from date palm and wheat were 26 and 33, respectively. All date palm samples were collected from one region, Muscat

Table 4 Pathogenicity of *Alternaria* species on date palm and wheat seedlings

Species name	Isolate code	Origin of isolates	% seedlings developing leaf spots within 10 days	
			Date palm	Wheat
<i>A. alternata</i>	DPM19	Date palm	10 b	70 a
<i>A. alternata</i>	WDK12	Wheat	30 ab	15 b
<i>A. slovaca</i>	WDK9	Wheat	70 a	35 ab
<i>A. slovaca</i>	WDK7	Wheat	40 ab	35 ab
<i>Alternaria</i> sp.	WDK11	Wheat	0 c	15 b
<i>Alternaria</i> sp.	DPM31	Date palm	30 ab	10 b
<i>A. jacinthicola</i>	WBR4	Wheat	30 ab	0 c
control	control		0 c	0 c

The sample sizes were 10 date palm seedlings and 20 wheat seedlings per isolate/control. Values with the same code in the same column are not significantly different from each other at $P < 0.05$ (Tukey's Studentized range test, SAS)

pathogenicity on date palm seedlings, with *A. slovaca* isolate being the most pathogenic, resulting in spots in 70% of the inoculated seedlings within 10 days of inoculation (Table 4). The isolate belonging to *A. burnsii* - *A. tomato* species complex from date palm produced necrotic spots on date palm leaves, but the other isolate from this species complex obtained from wheat did not produce any symptoms on date palm leaves. The control seedlings did not show any symptoms up to three weeks following inoculation.

Inoculating wheat seedlings with *Alternaria* species resulted in the production of necrotic spots, ranging in size from 1 to 5 mm by 1 - 3 mm in width (Fig. 1). Significant differences were observed in the pathogenicity of isolates on wheat (Table 4). The incidence of leaf spots caused by *A. alternata* isolate DPM19 from date palm was 70% compared to 15% produced by the *A. alternata* isolate WDK12 from wheat. Incidence of leaf spots varied from 10 to 35 by most of the other species. *A. jacinthicola* from wheat did not produce any symptoms on wheat leaves up to three weeks following inoculation (Table 4).

Discussion

Alternaria species have been reported as important pathogens of several crops (Gur et al. 2017; Moslemi et al.

2017; Abata et al. 2016). In this study, *Alternaria* species were the most common fungal species associated with leaf spots on date palm and wheat leaves.

Alternaria alternata has been reported as a main causative agent of leaf spot disease on many important plants such as pistachio in California (Avenot et al. 2016), *Aloe vera* in Oman (Al-Sadi et al. 2014), tobacco leaf spot (Duan et al. 2016) and black spot of apple (Tanahashi et al. 2016). Furthermore, *A. alternata* has been reported associated with leaf spot of date palm and wheat in Oman (Al-Sadi et al. 2012; Moghal et al. 1993). Findings from our current study showed that *A. alternata* was the most common *Alternaria* species associated with wheat leaf spot and it was also very common on date palm leaves. The isolates used for inoculation showed that they were pathogenic on both crops.

Alternaria burnsii was the most common pathogen on date palm and the second most common pathogen on wheat. *A. burnssi* has been reported from Rhizophoraceae, Menispermaceae, Malvaceae, Poaceae and Amaranthaceae (Woudenberg et al. 2015) and cause blight of cumin in Pakistan and Turkey (Shakir et al. 1995; Özer and Bayraktar 2015). *Alternaria tomato* is known to produce mycotoxins and cause leaf spot or nail-head spot on tomato (Shinha and Bhatnagar 1998). However, there are no reports of *A. burnssi* or *A. tomato* associated with wheat or date palm so far and this study appears to be the first report of isolates belonging to *A. burnsii* - *A. tomato* species complex causing leaf spot on wheat and date palms. Future studies of *A. burnsii* - *A. tomato* species complex may require additional loci to obtain a better separation of species.

Alternaria jacinthicola has been reported responsible for leaf blight disease on water hyacinth (*Eichhornia crassipes*) in Mali (Dagno et al. 2011). There are no previous records of association of *A. jacinthicola* with wheat. Our study showed that *A. jacinthicola* isolated from wheat did not cause any symptoms on wheat during pathogenicity tests, but the isolate used for inoculation caused leaf spot symptoms on 30% of the inoculated date palm seedlings. Therefore, *A. jacinthicola* may be present as a saprophyte on wheat or the isolate used for inoculation was not virulent on wheat. However, *A. jacinthicola* may be a potential pathogen on date palms. More isolates will be required to confirm this.

Alternaria infectoria (phylogenetically comprising *A. californica* and *A. slovaca*) has been recorded to

associate with leaf spot on wheat and this species is responsible for infection on many grain crops in Europe, Australia and Russia (Kahl et al. 2015; Perelló and Sisterna 2008). Our study reports *A. slovacica* associating with wheat leaf spot for the first time in Oman and this species was also found pathogenic on date palms.

Alternaria arborescens species complex is a common pathogen causing fruit rot of blueberry in California (Zhu and Xiao 2015), leaf blotch and fruit spot diseases of apple in Australia (Hartevelde et al. 2013), leaf spot on rice in Pakistan (Akhtar et al. 2014) and is a pathogen of several Solanaceae crops (Woudenberg et al. 2015). Previous studies showed that *A. arborescens* is associated with wheat leaf spot, but it was non-pathogenic on wheat (Mercado Vergnes et al. 2006). In Oman, Al-Sadi et al. (2012) recorded the association of *A. arborescens* with root rot and leaf spot on date palm where it was the most common species together with *A. alternata*. In our study, a single isolate of *A. arborescens* was obtained from date palm leaves developing leaf spot symptoms, but no pathogenicity test was conducted as the isolate was lost during preservation. Similarly, *A. ventricosa* and *A. caespitosa* were not included in the pathogenicity tests because they were lost during subculturing.

Characterization of *Alternaria* isolates based on the combined sequences of the TEF1, GPDH, RPB2, and ITS rDNA genes helped identify 59 *Alternaria* isolates to the species level. Additionally, *Alternaria* major allergen gene was used to identify species relationships in the section *Infectoriae*. The study showed the association of *Alternaria alternata*, *A. slovacica* and *A. burnsii* - *A. tomato* species complex with leaf spot in wheat. It also showed the association of *A. alternata* and *A. burnsii* - *A. tomato* species complex with leaf spot of date palm. Our study reports date palm and wheat as new hosts for *A. burnsii* - *A. tomato* species complex. It also reports *A. slovacica*, *A. ventricosa*, *A. caespitosa* and *A. burnsii* - *A. tomato* species complex for the first time in Oman. Also, one isolate belonging to *A. burnsii* - *A. tomato* species complex from date palm caused leaf spots on wheat and date palm leaves. This may suggest that *A. alternata* and *A. burnsii* - *A. tomato* species complex from date palm and wheat can cause cross infections. However, future studies should include more isolates from

both crops in order to investigate the degree of host specialization of *Alternaria* species on date palm and wheat.

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Compliance with ethical standards

Conflict of interest This is to confirm that this manuscript was not submitted for publication to any other journal. It is only submitted to this journal, EJPP. Authors have no conflict of interest.

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