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ORIGINAL ARTICLE

Genetic variability and host specialization in *Alternaria alternata* colonizing Solanaceous crops in Sudan

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Abstract

Early blight disease caused by *Alternaria* sp. is one of the most devastating diseases of Solanaceous crops widely distributed in Sudan. The aim of this study was to determine the genetic variation among different *Alternaria* isolates recovered from different Solanaceae crops showing typical symptoms of early blight disease. Infected leaves of tomato, potato, eggplant and pepper were collected from different geographical zones in Sudan. The recovered fungal isolates were identified to the genus level based on cultural and morphological characteristics. Five representative isolates were sent to the CABI Bioscience, U.K. for confirmation. The genetic relationship among the isolates was determined using the amplified fragments length polymorphism (AFLP) technique and the generated data were used to create similarity matrices using the PAST 3.01 software package. Dendrograms were constructed based on Jaccard's similarity coefficients. A total of 70 fungal isolates was recovered from the tested plants and all of them showed morphological characteristics typical of *Alternaria* spp. The conidia appeared in multiple-branched chains with spore sizes in the range of 2.38–13.09 µm × 12.30–43.63 µm. Therefore, the isolates were identified as *Alternaria alternata* (Fr.) Keissl. The identification was then confirmed by CABI.AFLP-based dendrogram which revealed five clusters with a significant cophenetic correlation coefficient ($r = 0.834$) between the dendrogram and the original similarity matrix irrespective of their geographical origins. Eighteen (75%) of the *Alternaria* isolated from tomato leaves were clustered together in cluster I and five isolates formed two separate clusters, *viz*. cluster IV (T-Kh5 and T-H1) and cluster V (T-H4 and T-Med2). The remaining isolate, T-Am5, grouped with one of the potato isolates in cluster III. The other isolates which were recovered from potato, pepper and eggplants were all separated from the tomato isolates in the largest cluster.

Keywords: amplified fragments length polymorphism (AFLP), *Alternaria*, diversity, early blight, Solanaceae

Introduction

Early blight disease, caused by *Alternaria solani* (Ellis & Martin) Jones & Grout, is one of the most common and destructive diseases of tomato and potato in areas of heavy dew rainfall and high relative humidity (Nash and Gardner 1988; Foolad *et al*. 2000; Kumar *et al*. 2008; El-Mougy 2009). *Alternaria tomatophila* has been proposed by Simmons (2000) as the common and widely distributed causal agent of early blight of tomato. Recently, Adhikari *et al*. (2017) reported several species of *Alternaria* as causal agents of early blight disease including *A. linariae* (which includes *A. solani* and *A. tomatophila*) and *A. alternata*.

The disease occurs under a wide range of climatic conditions and depends mainly on the frequency of foliage wetting from rainfall, fog, dew or irrigation, the nutritional status of foliage and cultivar susceptibility. The disease may produce rapid plant defoliation, collar rot on stems and fruit rot which can result in serious damage during all stages of development (Foolad *et al.* 2000). It is very difficult to manage early blight disease due to its broad host range, extreme variability among pathotypes or isolates and a prolonged active phase of the disease cycle (Chaerani and Voorrips 2006; Kumar *et al.* 2008). *Alternaria solani* has dark-coloured mycelium, and in older diseased tissues it produces short, simple, erect conidiophores that bear single or branched chains of conidia (Agrios 2005). The fungus produces large conidia which are broadest near the base and often gradually taper to an elongated beak (rostate), typically ovoid or obclavate, pale brown to brown, and multicelled, with transverse and frequently also longitudinal septa (Ellis and Ellis 1985; Rotem 1994; Thomma 2003). In 1985, Ellis and Ellis stated "*A. solani* is characterized by solitary, golden brown and beaked conidia that have 9–11 transverse septa and few or no longitudinal septa". The authors reported spore dimensions of 15–19 μm × 150–300 μm.

In Sudan, Tarr (1955) reported early blight disease in central and southern Sudan as well as on the Red Sea coast. The causal agent was determined as *A. solani*. Other solanaceous crops are also infected with the disease incited by *A. tenuis* (*A. alternata* Kessler) which is characterized by small spores of 6–13 μm × 25–53 μm (Rotem 1994) and an apical cluster of branching chains of the small conidia (Simmons 2007). But, according to Rotem (1994) and Tran-Dinh and Hocking (2004), the morphological classification of small-spored *Alternaria* spp. of which *A. alternata* is a member, is controversial. Isolates of *Alternaria* from tomato, apple, tobacco, citrus, pear and strawberry have been named variously as a single collective species called *A. alternata*, with distinct species, and as a different *forma especialis* or pathotypes e.g., *A. alternata* f. sp. *lycopersici* on tomato. In 1987, Giha reported that, two species of *Alternaria* were found attacking a variety of vegetables in Sudan including tomato, potato, eggplant and onion. These were *A. solani* and *A. tenuis*. *Alternaria solani* was the main species responsible for the early blight disease in the wetter parts of Sudan, while in the drier parts and in areas under irrigation *A. tenuis* was more predominant.

The genetic structure of field populations of *Alternaria* spp. was studied using molecular markers in *A. alternata* (Weir *et al.* 1998; Morris *et al.* 2000) and in *A. solani* (Weir *et al.* 1998; van der Waals *et al.* 2004; Weber and Halterman 2012). DNA fingerprinting was also used as a support of morphological taxonomy in *Alternaria* (Roberts *et al.* 2000). Few population genetic studies based on DNA markers addressed host specificity in *Alternaria* spp. (Kusaba and Tsuge 1994; Peever *et al.* 1999). Secondary metabolite profiling has been used to distinguish large-spore species e.g. *A. solani*. Metabolite cluster analysis of 56 isolates confirmed *A. dauci*, *A. solani* and *A. tomatophila* as three distinct species with their own metabolite profiles (Andersen *et al.* 2008). Taxonomic classification of the pathogen and characterization of its host specificity are important for understanding disease etiology and epidemiology and hence for disease management. Studies of the causal agent of early blight of solanaceous crops in Sudan are 40–60 years old. Furthermore, the assignment of the pathogen at species level was contradictory (Tarr 1955). The aim of this work was, therefore, to determine the fungal species responsible for early blight of tomato, potato, bell pepper and eggplant in Sudan, study their host specificity and characterize their populations.

Materials and Methods

Isolation and identification of the pathogen

Infected leaves of tomato, potato, eggplant and pepper crops showing typical symptoms of early blight disease were collected from different geographical regions representing northern, central and southern areas of Sudan. Segments from diseased leaves were washed thoroughly with tap water and surface sterilized with 5.25% sodium hypochlorite solution and plated onto Potato Dextrose Agar (PDA) medium supplemented with chloramphenicol (0.05 g · l^{-1}) as a bacteriostatic agent. Plates were incubated for 7 days at 25 ± 2°C and cultures were repeatedly subcultured for purification. Single spore cultures of 70 different *Alternaria* sub-cultures were prepared and maintained on PDA slants adopting Goh's (1999) technique. The pathogens were identified based on morphological characters, mainly spore sizes, pigmentation on the reverse side of the agar plate, growth patterns and spore chain formation. Discs (5 mm diameter) from 7-day-old cultures were placed on the surface of PDA plates. The plates were incubated at 25 ± 2°C for 9 days. The colony diameters were measured after 3, 6 and 9 days. Spore sizes were measured under a light microscope. Pigmentation was visually judged under daylight after 9 days. Five representative spore cultures: two tomato isolates (T1 {A-1} and T2 {A-2}), one potato isolate (P. {A-3}), one pepper isolate (Pe. {A-4}) and one eggplant isolate (E. {A-5}) were maintained on PDA slants and sent to the CABI Bioscience, the division of Commonwealth Agricultural Bureaux (CAB) International Centre, London U.K. for authentication.

DNA extraction and amplified fragments length polymorphism (AFLP) analysis

The DNA of the isolates was extracted following a CTAB protocol. The DNA amount was estimated by comparing bands in electrophoretic agarose gels with a known molecular weight DNA ladder. Then 100 ng DNA of each isolate was digested with *EcoRI* and *Tru*1I restriction enzymes. The digested DNA was ligated to the following adapters: EcoRI-Adapter 1 (CTCGTAGACTGCGTACC), EcoRI-Adapter 2 (AATTGGTACGCAGTC), Tru1I-Adapter 1 (TACT CAGGACTCAT), and Tru1I-Adapter 2 (GACGA TGAGTCCTGAG). Each reaction was prepared in 20 µl containing 10 µl DNA, 5 pmol of each adapter, T4-DNA-ligase-buffer and 1 µl T4DNA ligase (MBI Fermentas, Hilversum, Netherlands). The products were subjected to two-step amplification (Reineke and Karlovsky 2000); pre-selective and selective polymerase chain reaction (PCR). Two primers complementary to the adapter sequences with the addition of one selective nucleotide at the 3' end were used in the pre-selective PCR (Eco-A/Mse-C). Then four primers with three selective nucleotides (EcoR1--ACC, EcoR1-ACA, EcoR1-AGC and Mse1-CTA) were used in the selective amplification. The EcoRI-targeting primers used in the second amplification were labelled with fluorescent dyes Dy-750, Dy-681 and Dy-635 (Purimex, Grebenstein, Germany) compatible with a capillary sequencer CEQ8000 (Beckman Coulter, Krefeld, Germany). Amplified DNA fragments were denatured at 90°C for 120 s, loaded on the capillary sequencer and separated for 80 min at 4.8 kV. A set of size standards labelled with Cy5 (Standard 600, Beckman Coulter, Krefeld, Germany) was loaded onto each capillary together with samples to allow cross-capillary and cross-run comparisons. Peaks of the electropherogram were converted into a binary matrix and pairwise similarity coefficients were calculated according to the Jaccard similarity index. UPGMA (unweighted pair-group method using arithmetic averages) ordination was carried out based on this similarity matrix using PAST vn. 3.1 software (Rohlf 2000). The cophenetic correlation coefficient was calculated to test how well the dendrogram represented the corresponding data matrix (Romesburg 2004). Dendrograms were also constructed for isolates recovered from each crop to detect genetic variation within groups.

Cross infectivity

This experiment was conducted during the winter of 2009/2010 to study the cross infectivity of *A. alternata* (Fr.) Keissl. isolates within tomato, potato, pepper and eggplant. Seeds of tomato (Peto 86), potato (Ajaxa), eggplant (Black beauty) and pepper (California wonder) were sown in seedbeds containing sterilized peat moss soil. Four weeks later, seedlings of each crop were transferred to metal planter pots each containing 1 kg sterilized beat moss. Five pots, each with two seedlings of each crop, were kept in a separate cage as one treatment. Inoculations with the five representative spore culture suspensions (mentioned above) were made when the plants were six-weeks-old by spraying the inocula with a mini-high pressure sprayer. Plants in each cage were inoculated with one of the isolates, while the plants in the tenth cage were inoculated with sterilized distilled water as a control treatment. The cages were then placed parallel to each other in an east-west direction.

The response of the inoculated plants was recorded three times during the growing season. An arbitrary disease severity rating scale 0–4 was adopted following Horsfall and Barratt scale (Bock *et al.* 2009). In this scale, 0 = healthy plants (no visible disease symptoms), 1 = lesions occupying one quarter of the area of infected leaves, 2 = lesions occupying half the area of infected leaves, 3 = lesions occupying three quarters of the area of infected leaves, 4 = lesions occupying almost the entire leaf area.

Results

Identification of early blight causal agent

The identification of the fungus was based on the morphological characters. The cultures of the 70 isolates were characterized by a slow to medium growth pattern on PDA. Cultures on PDA initially turned fluffy and off-white, with various shades of green and brown and also pink. Later, most of the colonies became appressed and nearly black. Microscopic examination at ×40 magnification for all the isolates showed some variations in shape and size of the conidia. The conidia were multicellular, pale brown to brown with or without elongated beaks, giving a club-like appearance. The length of spores isolated from infected tomato leaves was 5.55–43.63 µm with a mean of 22.51 µm, while the width of spores in the widest part of the conidium ranged from 2.38 µm to 13.09 µm with an average of 6.95 µm. The dimensions of spores isolated from infected potato leaves were 7.14–13.49 µm × 11.9–32.53 µm with an average of 9.70 × 21.82 µm. The dimensions of spores from pepper plants were 9.52–11.90 × × 22.21–34.11 µm and the average was 10.71 × 27.66 µm. Spores isolated from infected eggplant leaves had dimensions ranging from 6.35–12.30 µm to 23.01–34.91 µm with an average of 9.96 × 29.09 µm (Tables 1–4). The examined conidia varied in beak formation; some of them were beaked while others were

not. Cultures were examined under a stereomicroscope for chain formation. Chains consistently appeared in all cultures. Thus, the causal agent of early blight disease was identified as *A. alternata* (Fr.) Keissl.

The CABI identification report (CABI Report) confirmed that the five representative isolates (T1 with IMI No. of 398172, T2 with IMI No. 398173, P with IMI No. 398174, Pe with IMI No. 398175, and E with IMI No. 398176) which were isolated from infected leaves displaying typical early blight disease symptoms of tomato, potato, pepper and eggplant, respectively, were *A. alternata* (Fr.) Keissl.

Genetic variation between the isolates

The UPGMA dendrogram constructed from AFLP data for all of the 70 isolates, recovered from the four crops, is shown in Figure 1. The cophenetic correlation coefficient was 0.83376, indicating that the dendrogram represents pairwise genetic distances between strains fairly well. The geographical origins did not affect the distribution of the isolates within the cluster. As seen in this Figure, 18 (75%) of *Alternaria* isolated from tomato leaves were clustered together in cluster I and five isolates formed two separate clusters, viz. cluster IV (T-Kh5 and T-H1) and cluster V (T-H4 and T-Med2). The remaining isolate, T-Am5, grouped with one of the potato isolates in cluster III. The other isolates which were recovered from potato, pepper and eggplants were all separated from the tomato isolates in the largest cluster (cluster II).

Dendrograms constructed for the isolates of each individual crop are shown in Figures 2–5. The cophenetic correlation coefficients were 0.916 (tomato),

Table 1. Morphological description of *Alternaria alternata* isolated from tomato leaves

Isolates	Colour of the cultures	Linear growth [mm]			Spore size [µm]	Length of beaks [µm]
		72 h	144 h	216 h		
T-kh$_2$	Pale green	35	57	58	10.31 × 43.63	0–2.38
T-kh$_3$	Brown	32	51	68	7.14 × 28.56	0–0.79
T-kh$_5$ I	Grey	32	47	65	2.38 × 5.55	0–1.59
T-kh$_6$ I	Greyish green	31	55	77	6.35 × 20.63	0.00
T-kh$_6$ II	Pale green	36	55	70	9.52 × 34.91	0–4.76
T-H$_2$ I	Light green	31	50	70	7.14 × 18.25	0.00
T-H$_2$ II	Light green	30	50	73	5.55 × 10.31	0.00
T-H$_3$ I	Brown	29	47	65	9.52 × 26.18	0–3.97
T-H$_3$ II	Brown	30	49	67	9.52 × 21.42	0–0.79
T-H$_4$	Brownish green	29	49	72	8.73 × 21.42	0.00
T-H$_5$ I	Brown	31	52	70	9.52 × 21.42	0–0.40
T-H$_5$ II	Light brown	32	50	61	9.52 × 27.77	0–0.79
T-Am$_1$ I	Brown	29	45	60	8.73 × 19.04	0–1.59
T-Am$_1$ II	Brown	29	43	57	10.31 × 22.21	0–6.35
T-Am$_1$ III	Brownish green	24	39	49	8.73 × 19.83	0.00
T-Am$_2$ I	Brown	27	43	58	9.52 × 20.63	0.00
T-Am$_2$ II	Brown	30	51	72	7.14 × 20.63	0–3.97
T-Am$_3$	Green	31	49	63	13.09 × 27.77	0–1.59
T-ka$_1$	Green	30	52	70	11.11 × 28.56	0–4.76
T-ka$_2$	Brownish green	32	48	57	9.52 × 23.01	0–1.19
T-ka$_3$ I	Green	30	53	75	11.9 × 26.18	0.00
T-ka$_3$ II	Green	33	58	78	10.31 × 26.18	0–3.17
T-ka$_4$	Light brown	32	56	73	11.11 × 29.35	0–3.97
T-ka$_5$	Brown	32	53	72	9.52 × 19.04	0–0.79
T-med$_1$	Green	25	39	52	7.14 × 22.21	0–3.17
T-med$_2$	Green	26	41	50	11.11 × 23.01	0–1.19

T – Tomato; Am – the location Amrey; kh – the location Khartoum; H – the location Hajer Alasal; med – the location Wed Madani

Table 2. Morphological description of *Alternaria alternata* isolated from potato leaves

Isolates	Colour of the cultures	Linear growth [mm]			Spore size [µm]	Length of beaks [µm]
		72 h	144 h	216 h		
P-sh$_2$ I	Light brown	31	42	43	7.14 × 18.25	0–2.38
P-sh$_2$ II	Brownish green	29	46	63	11.11 × 20.63	0–0.79
P-sh$_2$ III	Brownish green	31	43	45	11.11 × 22.21	0–1.59
P-sh$_3$ II	White	31	56	73	8.33 × 16.66	0.00
P-sh$_3$ III	Light grey	29	48	69	8.73 × 32.53	0–4.76
P-sh$_4$ I	Green	30	55	78	11.11 × 28.56	0.00
P-sh$_4$ II	Brownish green	30	51	74	10.31 × 23.01	0.00
P-sh$_4$ III	Brownish green	32	56	78	8.73 × 17.45	0–3.97
P-sh$_6$ I	Light brown	34	53	71	7.93 × 21.42	0–0.79
P-sh$_6$ II	Light brown	32	49	51	7.93 × 16.66	0.00
P-kh$_1$ I	Light green	33	59	80	10.31 × 26.18	0–0.40
P-kh$_1$ II	Pinkish white	33	50	66	7.14 × 11.90	0–0.79
P-kh$_1$ III	Pale green	31	51	72	7.93 × 17.45	0–1.59
P-kh$_1$ IV	Light brown	29	45	67	11.9 × 25.39	0–6.35
P-kh$_1$ V	Pale green	30	49	65	13.49 × 27.77	0.00
P-kh$_2$ I	Brownish green	30	51	72	7.93 × 23.01	0.00
P-kh$_2$ II	Light green	29	44	56	9.52 × 16.66	0–3.97
P-Am$_1$ I	Green	29	45	65	11.90 × 23.80	0–1.59
P-Am$_1$ II	Brown	29	46	61	10.31 × 19.83	0–4.76
P-Am$_1$ III	Brownish green	31	46	62	10.31 × 15.87	0–1.19
P-Am$_1$ IV	Light green	29	50	79	9.52 × 21.42	0.00
P-Am$_2$ I	Brownish green	26	40	56	11.11 × 19.83	0–3.17
P-Am$_2$ II	Brownish green	27	45	62	7.93 × 27.77	0–3.97
P-Am$_2$ III	Brownish green	30	51	72	11.11 × 29.35	0–0.79

P – potato; Am – the location Amrey; kh –the location Khartoum; sh – the location Alshehainab

Table 3. Morphological description of *Alternaria alternata* isolated from pepper leaves

Isolates	Colour of the cultures	Linear growth [mm]			Spore size [µm]	Length of beaks [µm]
		72 h	144 h	216 h		
Pe-H$_1$ I	Pale yellowish green	34	62	77	10.31 × 23.01	0–5.55
Pe-H$_1$ II	Pale yellowish green	35	62	78	11.11 × 30.15	0–4.36
Pe-H$_2$ I	Pale whitish green	27	50	68	11.90 × 22.61	0–0.79
Pe-H$_2$ II	Pale whitish green	30	51	69	9.52 × 22.21	0–6.35
Pe-H$_2$III	Pale green	32	51	61	11.11 × 25.39	0–4.76
Pe-H$_2$ IV	Pale green	29	50	61	11.50 × 24.59	0–1.59
Pe-H$_3$ I	Green	32	56	79	11.90 × 34.11	0–3.17
Pe-H$_3$ II	Light brown	31	52	76	9.52 × 31.73	0–9.52
Pe-H$_3$ III	Pale yellowish green	31	59	77	10.31 × 31.73	0–3.97
Pe-H$_3$ IV	Pale green	33	60	61	9.52 × 30.15	0–6.35
Pe-H$_3$ V	Pale green	30	57	75	11.11 × 28.56	0–5.55

Pe – pepper; H – the location Hajer Alasal

Table 4. Morphological description of *Alternaria alternata* isolated from eggplant leaves

Isolates	Colour of the cultures	Linear growth [mm]			Spore size [µm]	Length of beaks [µm]
		72 h	144 h	216 h		
E-Am_1	Green	35	62	80	11.90 × 34.91	0–7.14
E-Am_2 I	Green	28	56	73	9.52 × 26.18	0–7.14
E-Am_2 II	Green	35	62	77	11.11 × 34.11	0–11.9
E-H_1 I	Pale yellowish green	32	50	66	12.30 × 30.15	0–5.55
E-H_1 II	Green	30	56	77	10.71 × 23.80	0–3.57
E-H_1 III	Pale yellowish green	26	55	75	10.31 × 24.59	0–4.76
E-H_2	Pale yellowish green	26	45	67	7.14 × 33.32	0–5.55
E-H_3 I	Yellowish green	28	45	65	0.31 × 31.73	0–4.76
E-H_3 II	Pale yellowish green	29	52	83	6.35 × 23.01	0–2.38

E – eggplant; Am – the location Amrey; H – the location Hajer Alasal

Table 5. Susceptibility of tomato, potato, pepper and eggplant inoculated with five isolates of *Alternaria alternata*

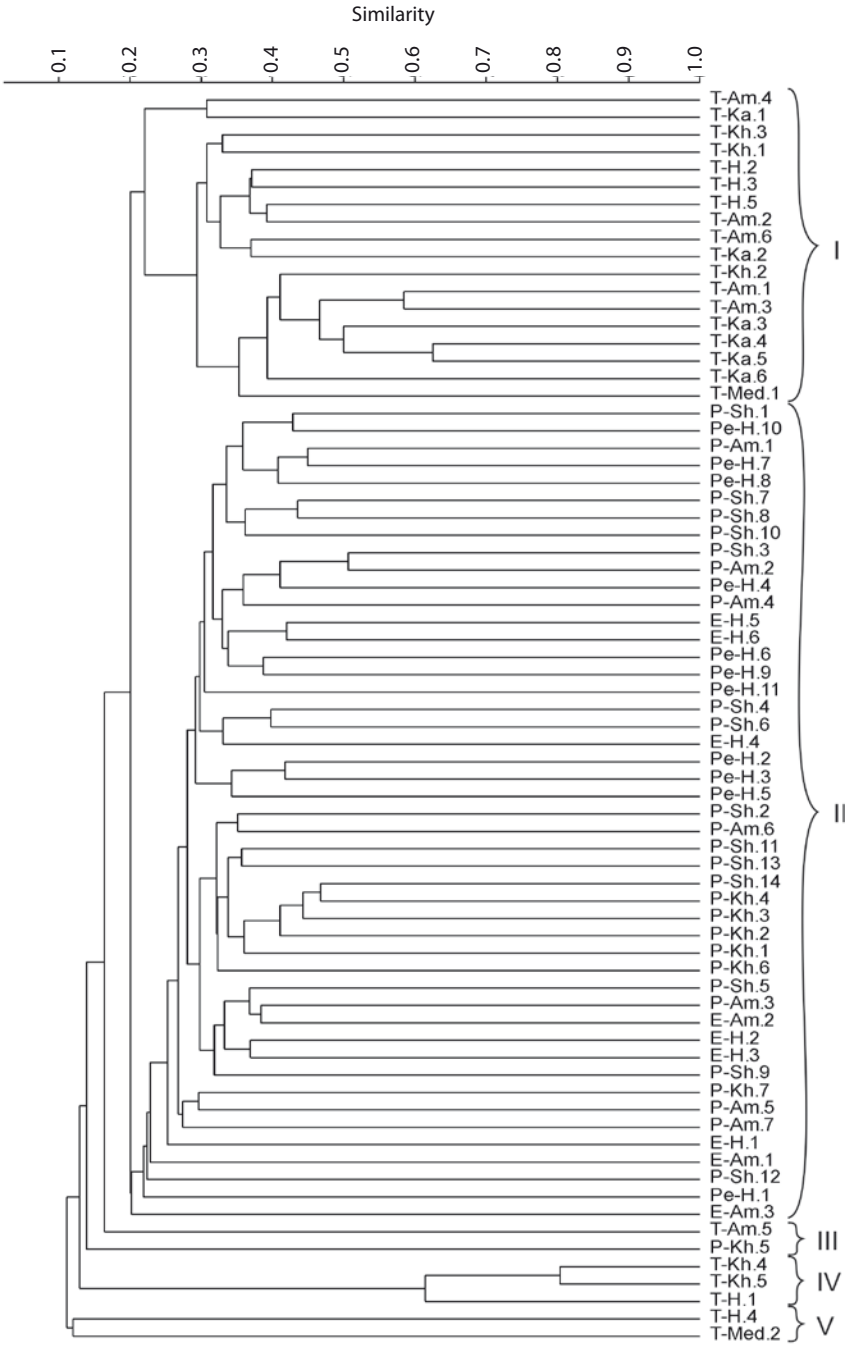
Isolates	Weeks after inoculation	Response of inoculated plants [severity %]			
		tomato	potato	pepper	eggplant
E	Two weeks	25	25	0	0
Pe		25	25	0	0
P		25	25	0	0
T_1		25	25	0	0
T_2		25	25	0	0
Control		0	0	0	0
E	Six weeks	75	75	0	0
Pe		75	75	50	0
P		75	75	25	0
T_1		75	75	0	0
T_2		75	75	50	0
Control		0	0	0	0
E	Seven weeks	75	75	0	25
Pe		75	75	50	50
P		75	75	50	25
T_1		75	75	50	50
T_2		75	75	50	25
Control		0	0	0	0

E – eggplant, Pe – pepper, P – potato, T – tomato

0.739 (potato), 0.846 (pepper) and 0.873 (eggplant). Figure 2 indicates that the highest similarity (80%) among the tomato isolates was detected for T-Kh.4 and T-Kh.5 while the lowest similarity was recorded for T-Kh5 and T-Am4 (6%).

Host specialization

Results of cross infectivity are shown in Table 5. The characteristic symptoms of early blight disease were first observed 15 days after inoculation (the plants were 8-weeks-old) on all potato and tomato plants inoculated with one of the five *A. alternata* isolates recovered from the four crops. On the other hand, no symptoms were observed on peppers and eggplants. Table 5 also reveals that the inoculated eggplant seedlings remained symptomless 6 weeks after inoculation after which they started to show symptoms with 25–50% severity. In the seventh week after inoculation, almost all the plants of the four crops were infected.



*Cophenetic correlation coefficients r = 0.83376

Fig. 1. The phylogenetic tree of *Alternaria* species isolated from infected leaves of tomato, potato, pepper and eggplants collected from different parts of Sudan

Pepper plant seedlings never showed infection when inoculated with the isolates recovered from eggplants. Symptoms observed on potato and tomato plants steadily developed. Plants in the control treatment remained healthy throughout the duration of the experiment.

Discussion

Depending on the laboratory investigations the causal agent of early blight disease in tomato, potato, pepper and eggplant crops in Sudan is *A. alternata* (Fr.) Keissl. Identification was based on spore dimensions, including the beak which is considered as the most important characteristic of a particular *Alternaria* species. Spore sizes of the isolates ranged between 2.38–13.09 × 12.30–43.63 µm. These measurements agreed precisely with published descriptions of *A. alternata* (Rotem 1994) and differed from reported measurements for *A. solani*; 15–19 × 150–300 µm (Ellis and Ellis 1985) and 12–20 × 67–178 µm (Rotem 1981).

The CABI identification report confirmed that the causal agent of early blight disease in solanaceous crops

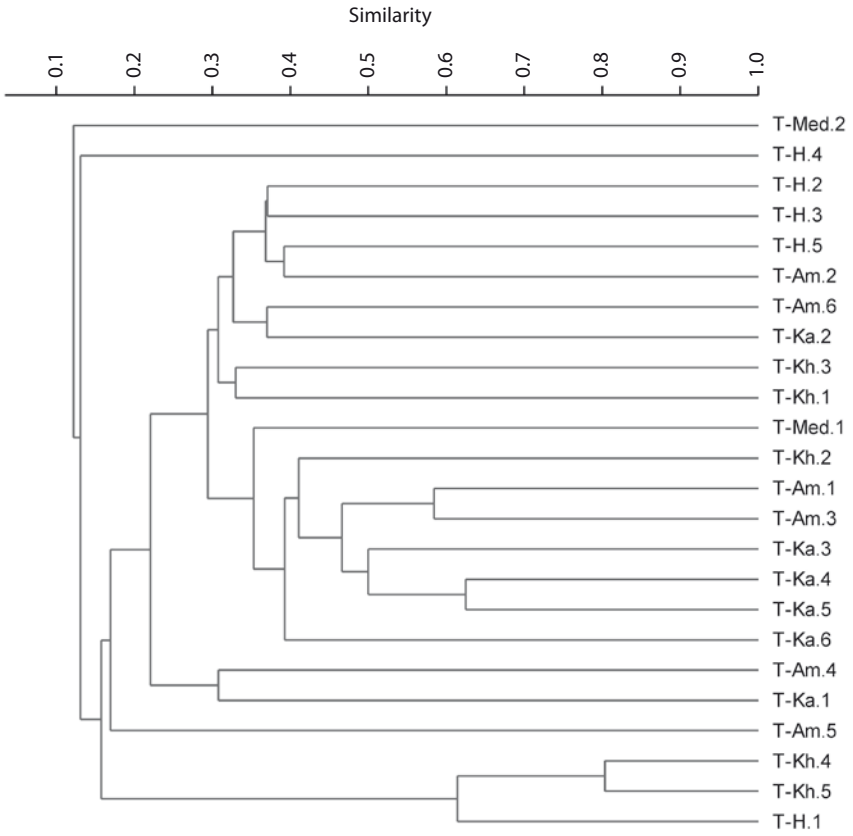


Fig. 2. The phylogenetic tree of *Alternaria* species isolated from infected leaves of tomato plants collected from different parts of Sudan. Cophenetic correlation coefficients r = 0.916

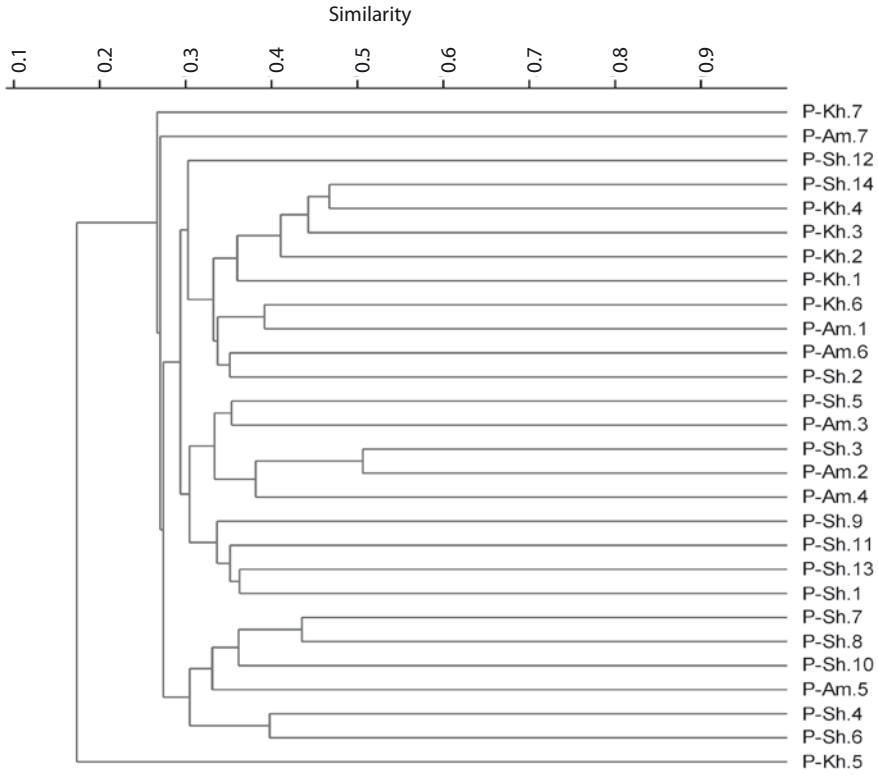


Fig. 3. The phylogenetic tree of *Alternaria* species isolated from infected leaves of potato plants collected from different parts of Sudan. Cophenetic correlation coefficients r = 0.739

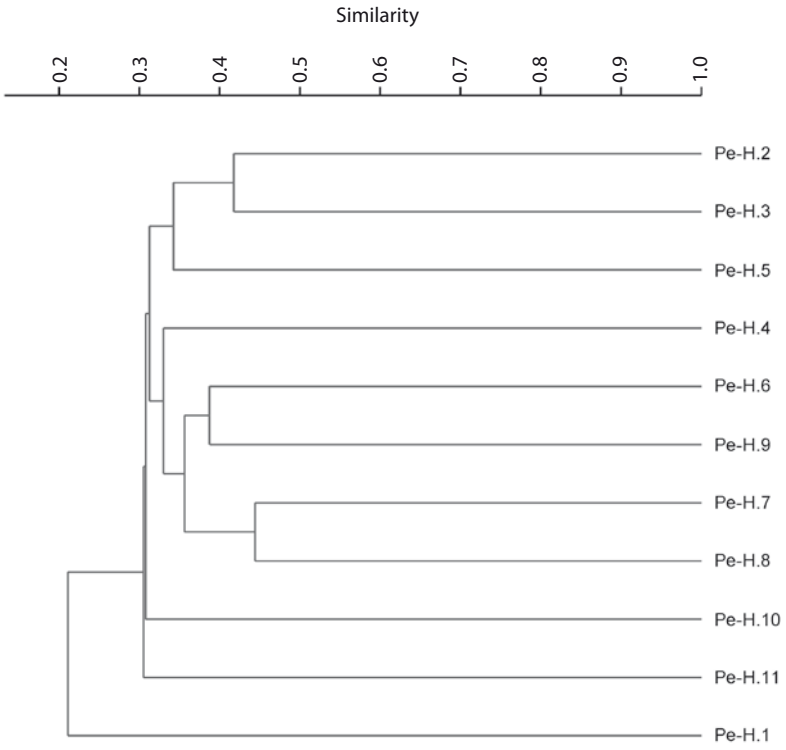


Fig. 4. The phylogenetic tree of *Alternaria* species isolated from infected leaves of pepper plants collected from different parts of Sudan. Cophenetic correlation coefficients r = 0.846

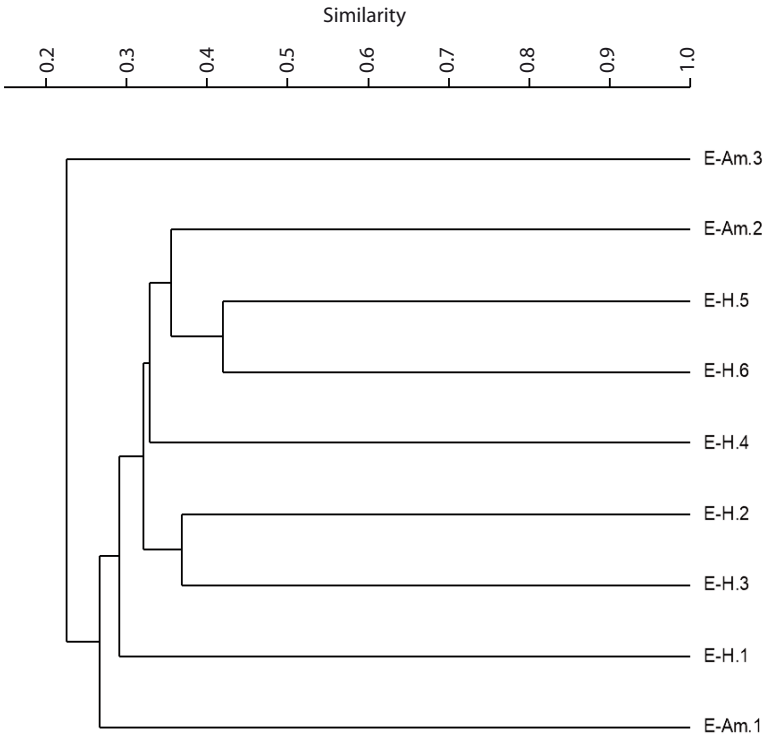


Fig. 5. The phylogenetic tree of *Alternaria* species isolated from infected leaves of eggplant collected from different parts of Sudan. Cophenetic correlation coefficients r = 0.873

in Sudan is *A. alternata* and settled the debate about the causal pathogen of early blight disease (CABI report).

The population structure of the pathogen and the genetic differences among strains isolated from different crops were studied by AFLP. *Alternaria alternata* strains isolated from tomato were genetically distinct from strains isolated from potato, pepper and eggplant. These results corroborated the establishment of forma specialis *lycopersici* specific for tomato (Rotem 1994; Tran-Dinh and Hocking 2004) and indicated that differentiation of the strain`s genome adapted to tomato plants paralleled their physiological specialization. Interestingly, AFLP patterns did not distinguish between isolates from potato, pepper and eggplants. Apparently the host range did not play a role in the genetic differences between these isolates which is in agreement with Rotem (1994) who stated that "the variability of strains of *Alternaria* species is not always associated with their host ranges". Also, these findings concurred with a previous study using RAPD-PCR (random amplification of polymorphic DNA-PCR) to measure the degree of genetic variation among isolates of *A. alternata* and *A. solani* isolated from potato and tomato by Weir *et al.* (1998). The geographic distribution of the *A. alternata* isolates had no correlation with genetic markers. These findings agreed with Perez-Martinez *et al.* (2004) who analysed 112 isolates of *A. solani* from diverse countries by AFLP and found that there was no significant correlation between genetic markers and geographic origin of the isolates.

Early blight disease symptoms were observed 2 weeks after inoculation on tomato and potato plants only, and no symptoms were observed on pepper and eggplant. Then, disease symptoms steadily developed with time on tomato and potato plants in all treatments. Six weeks after inoculation, disease symptoms began to appear on pepper plants. On eggplant, early blight disease symptoms were observed 7 weeks after inoculation. The symptoms observed on pepper and eggplant were mild. These results are compatible with Tarr (1955) who reported that other solanaceous crops besides tomato were also infected with early blight disease. These results show that early blight disease can also cause damage on potato, pepper and eggplant (Hansen 2009), and agree with Rotem (1994) and Robert (1999). It is apparent that the isolates of *A. alternata* used in this experiment were capable of infecting the four solanaceous crops with different levels of severity. The symptoms observed on pepper and eggplant were mild in comparison with those on tomato and potato. These observations proved that early blight is a serious disease on tomato and potato crops but rarely affects pepper and eggplant. Furthermore, pepper and eggplant are not important hosts for the pathogen (Reiner *et al.* 2004). The severe symptoms on tomato and potato plants and the mild symptoms on pepper and eggplant can be ascribed to the virulence variability of the strains of this pathogen, agreeing with Rotem (1994), who stated "the variability of strains of *Alternaria* species is not always associated with their host ranges. *Alternaria alternata* typically displays differences; such difference can be in virulence". Early blight disease in pepper and eggplant crops had not been studied in Sudan and the pathogenic differences have yet to be examined for all solanaceous crops. The cross infectivity of *A. alternata* among tomato, potato, eggplant and pepper crops which was studied in this experiment obviously showed that there are not different isolates of *A. alternata* specific on these different crops. The same isolate of the pathogen can attack and cause early blight symptoms on each of these four crops. These findings should be considered in any disease management strategy. Tomato, eggplant and pepper crops are cultivated throughout the year in Sudan. They are sown at different seasons i.e. rainy season and winter; in different regions. Potato is grown in winter in northern Sudan. In western Sudan, mainly Jabel Marra, it is grown in winter and rainy seasons. This situation entails the availability of the inocula of *A. alternata* throughout the year endangering the cultivated solanaceous crops. The situation is aggravated by the fact that so far no source of resistance has been detected in varieties and cultivars of potato, tomato, eggplant and pepper in Sudan. Thus, comprehensive and practical management strategy to be implemented should consider that *A. alternata* is a real threat to the production of solanaceous crops all year round across the country.

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