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The Incidence of *Alternaria* Species Associated with Infected *Sesamum indicum* L. Seeds from Fields of the Punjab, Pakistan

Brian Gagosh Nayyar[1]*, Steve Woodward[2], Luis A. J. Mur[3], Abida Akram[1], Muhammad Arshad[1], S. M. Saqlan Naqvi[4], and Shaista Akhund[1]

[1]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46300, Pakistan*
[2]*Institute of Biological and Environmental Sciences, University of Aberdeen, Cruikshank Building, St. Machar Drive, Aberdeen AB24 3UU, Scotland, UK*
[3]*Institute of Biological, Rural and Environmental Sciences, Aberystwyth University, Edward Llwyd Building, Penglais Campus, Aberystwyth SY23 3DA, Wales, UK*
[4]*Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi 46300, Pakistan*

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Sesame (*Sesamum indicum*) is an important oil seed crop of Asia. Yields can be negatively impacted by various factors, including disease, particularly those caused by fungi which create problems in both production and storage. Foliar diseases of sesame such as *Alternaria* leaf blight may cause significant yield losses, with reductions in plant health and seed quality. The work reported here determined the incidence of *Alternaria* species infecting sesame seeds grown in the Punjab, Pakistan. A total of 428 *Alternaria* isolates were obtained from 105 seed samples and grouped into 36 distinct taxonomic groups based on growth pattern and morphological characters. Isolation frequency and relative density of surface sterilized and non-surface sterilized seeds showed that three isolates (A13, A47 and A215) were the most common morphological groups present. These isolates were further identified using sequencing of the Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA) and the *Alternaria* major allergen gene (*Alt a 1*). Whilst ITS of rDNA did not resolve the isolates into *Alternaria* species, the *Alt a 1* sequences exhibited > 99% homology with *Alternaria alternata* (KP123850.1) in GenBank accessions. The pathogenicity and virulence of these isolates of *Alternaria alternata* was confirmed in inoculations of sesame plants resulting in typical symptoms of leaf blight disease. This work confirms the identity of a major source of sesame leaf blight in Pakistan which will aid in formulating effective disease management strategies.

Keywords **:** *Alternaria*, *Alt a 1* gene, ITS, Punjab, sesame seeds

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Sesame (*Sesamum indicum* L.) is one of the oldest and most important oilseed crops, mainly grown for its oil and protein content (Johnson et al., 1979). This crop is an important source of fats, proteins, vitamins and minerals in the food of rural people, particularly children, throughout the world (Shahidi et al., 2006). Sesame seed contains oil (48 to 60%), protein (18 to 23.5%), carbohydrate (13.5%), and ash (5.3%), with a moisture content of approximately 5.2% (Obiajunwa et al., 2005; Kahyaoglu and Kaya, 2006). Sesame oil contains the antioxidants sesamoline, sesamin and sesamol (Pastorello et al., 2001). Sesame oil is used in cuisine for salad dressings and the manufacturing of margarine, and is a raw ingredient in industry for making paints, varnishes, soaps, perfumes, insecticides, and pharmaceuticals as a vehicle for drug delivery (Grubben and Denton, 2004).

Cultivation of sesame probably originated in the Harappa Valley region of the Indian subcontinent as long as ago as

*Corresponding author.
Phone) +92-334-809-8095, FAX) +92-51-9290160
E-mail) brian_gagosh@hotmail.com

pre-3000 BC (Ashri, 2007). Currently, sesame is grown commercially in 76 countries in the world but production is mostly dominated by Asian and African countries (Ashri, 1998). India, Sudan and China are the leading producers of sesame, followed by Myanmar, Nigeria, Ethiopia, Uganda, Mexico, Pakistan, Bangladesh, Thailand, Turkey (FAO, 2014). In Pakistan, sesame is grown in all four provinces, but 89% of production is in the Punjab, which is considered a major sesame producing area in South Asia (The Financial Daily, 2017).

Numerous microorganisms, especially fungi, pose a challenge to both sesame production and seed storage. Sesame agriculture faces numerous disease problems, including vascular wilt, root rot, leaf blight, leaf spot and damping off in young seedlings (Farhan et al., 2010). Previous reports have shown the presence of *Aspergillus* and *Fusarium* in sesame seeds (Mbah and Akueshi, 2000, 2001). However, sesame is also susceptible to leaf blight caused by *Alternaria sesami* (Kolte, 1985), which is also seed-borne (Richardson, 1979). Leaf diseases of sesame, such as blight diseases caused by *Alternaria* spp. result in substantial loss in yield and deterioration in quality and vigor of seed (Enikuomehin et al., 2002). Of particular economic importance is leaf blight caused by *Alternaria sesami* and *A. alternata* (Chohan, 1978), which also causes seed rot and pre- and post-emergence damping-off, as well as infecting all aerial plant parts, resulting in considerable losses in yield, both qualitative and quantitative (Naik et al., 2004). *Alternaria* leaf spot of sesame has been recognized as a major biotic pressure of single origin limiting yields (Lubaina and Murugan, 2013). Symptoms of *Alternaria* leaf blight include the formation of round to irregular spots of up to 10 mm diameter. Individual spots may coalesce to form large necrotic patches, resulting in premature abscission (Rao and Vijayalakshmi, 2000). *Alternaria* species are dispersed from region to region through various pathways which include air-borne conidia and adherence of soil to seedlings, farm equipment, or animals (Ojiambo, 1997).

Alternaria species produce non-host specific (e.g., tenuazonic acid (TeA), alternariol (AOH), alternariol monomethyl ether (AME), brefeldin A, tentoxin, zinniol) (Saha et al., 2012) as well as host-specific toxins (Thomma, 2003) which contaminate the product. Consumption of foodstuff contaminated with *Alternaria* toxins has been implicated in elevated incidence of esophageal carcinoma in humans (Schrader et al., 2001). The wide distribution, high variability and influence of *Alternaria* spp. on crops and humans warrants accurate identification of the causal agents to formulate effective control and management strategies (Hong et al., 2005b).

The aim of the work described in this paper was to determine the *Alternaria* species associated with sesame in the Punjab, Pakistan, as an example of the source of yield losses affecting production of this important crop in South Asia. To the best of our knowledge, no such studies have been carried out to date in the Punjab. This basic work on testing seeds for the presence of seed-borne pathogens is an important step in the development of disease management strategies for this crop.

Materials and Methods

Plant Material and Fungal Isolation. One hundred and five seed samples of sesame were collected from the major sesame growing areas of the Punjab, Pakistan (Fig. 1). Seeds for isolating fungi were selected randomly from within a batch and 50% of seeds were surface sterilized in 5% NaOCl for 2 mins. Surface sterilized and unsterilized seeds were placed at the rate of 25 seeds into 90 mm diameter Petri dishes with three layers of well-moistened filter paper discs (Whatman™ 1001-090 Grade 1). Petri dishes were incubated at 22 ± 2ºC for seven days with an alternate cycle of light and darkness (12 h each) in a Versatile Environmental Test Chamber (Sanyo, Japan); illumination was provided by 55W fluorescent lights (Sylvania, Germany) giving a light intensity of 125-130 µmol m^{-2} s^{-1}. The experiment was performed in triplicate. After incubation, emerging fungal colonies were counted and isolated onto potato dextrose agar (PDA, Oxoid, UK) amended with 100 mg L^{-1} streptomycin (Singleton et al., 1993). Isolates were maintained on PDA and identified on the basis of morphological characters (Ellis, 1971, 1976; Simmons, 2007).

Isolation frequency (Fr) and relative density (RD) of fungi in and on seeds were calculated as follows:

$$\text{Fr}\,(\%) = \frac{(\text{ns})}{\text{N}} \times 100 \qquad \text{RD}\,(\%) = \frac{(\text{ni})}{\text{N}} \times 100$$

Where, ns is the number of samples on which a fungus occurred; N is the total number of seeds sampled; ni is the number of isolates of a fungal genus/species, and Ni is the total number of fungal isolates obtained.

Fungal DNA Extraction, PCR amplification, and Sequencing. Genomic DNA of representative isolates of the three most frequent groups of *Alternaria* (A13, A47, and A215) was extracted by scraping the surface of 14 days old cultures grown on PDA. DNA was extracted from 50 mg mycelium per isolate using "Plant DNeasy Mini Kit" (Qiagen, UK) by homogenizing in liquid nitrogen and following the manufacturer's protocol. DNA concentra-

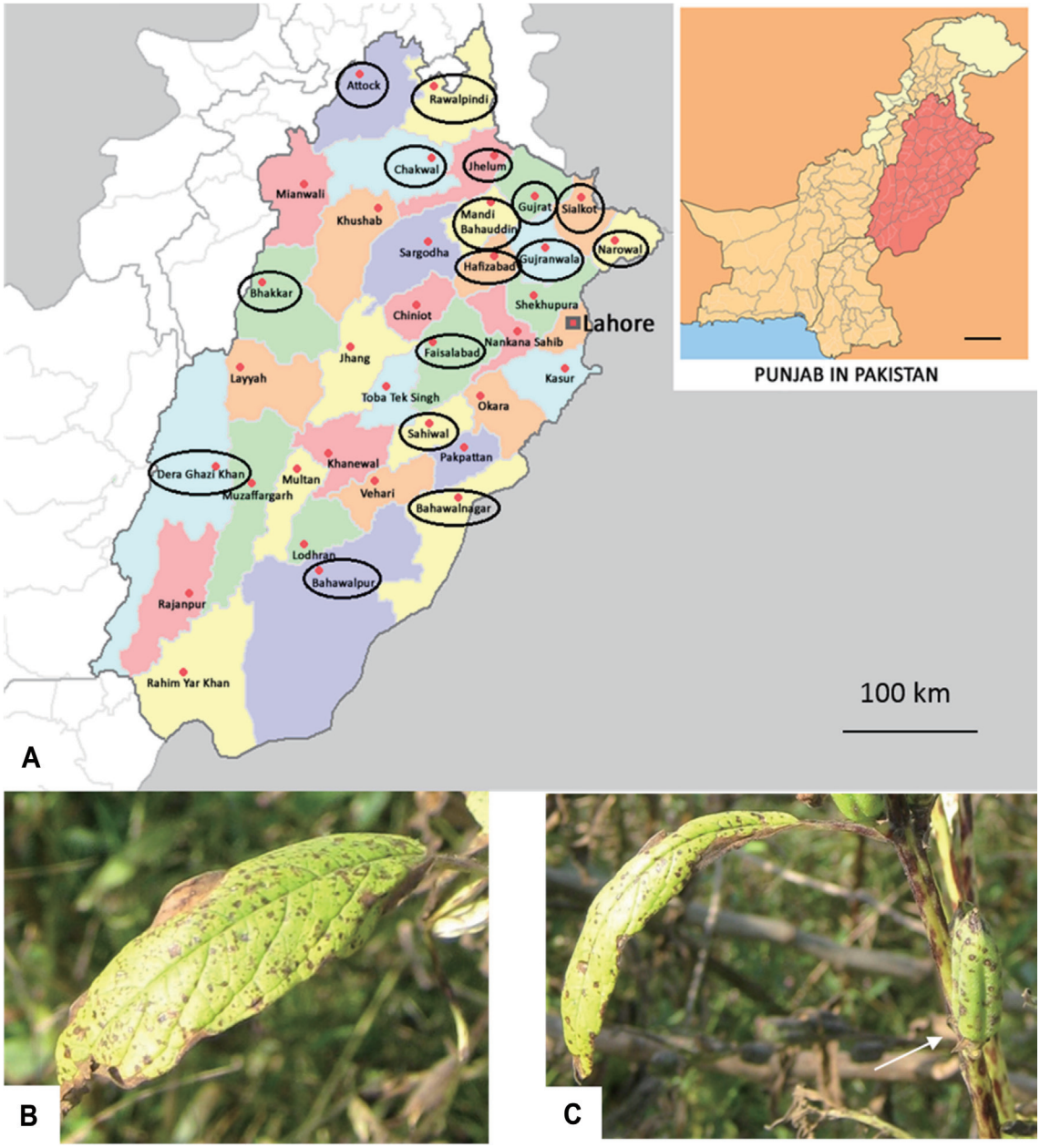

Fig. 1. (A) Sampling sites in the Punjab, Pakistan. (B) Typical leaf blight symptoms on sesame leaves in the field and (C) pods (arrowed) during the sampling in the Punjab, Pakistan.

tion was measured by a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies Inc., Montchanin, DE, USA) and the final concentration of DNA was adjusted to 100 ng/µl for PCR amplification. The Internal Transcribe Spacer (ITS) region of rDNA and *Alt a 1* gene were amplified using ITS1 (TCCGTAGGTGAACCTGCGG) / ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990) and Alt-for (ATGCAGTTCACCACCATCGC) / Alt-rev (ACGAGGGTGAYGTAGGCGTC) primers (Hong et al., 2005a), respectively.

PCR was performed in a 25 µl reaction mixture containing 12.5 µl BioMix (Bioline), 0.5 µl each primer, 2 µl template DNA and 9.5 µl pure water. PCR was performed in a MyCycler™ Thermal cycler (Bio-Rad, USA) with initial denaturation at 95ºC for 3 mins followed by 35 cycles of 94ºC for 30s, 55ºC for 30 s and 72ºC for 1 min and a final elongation step at 72ºC for 10 min for ITS amplification. For the *Alt a 1* gene amplification, an initial denaturation at 94ºC for 1 min was followed by 35 cycles of 94ºC for 30 s, annealing at 57ºC for 30 s, 72ºC for 1 min, and a final

extension at 72ºC for 10 min. Amplified fragments were loaded on an agarose gel (1% w/v) stained with SYBR® Safe DNA gel stain (Invitrogen, USA), visualized under UV light and purified with a "Qiaquick PCR Purification kit" (Qiagen, UK), following the manufacturer's protocol. Amplicons were sequenced by Source BioScience (UK) in both directions and the sequences analyzed using MEGA 7 software (Kumar et al., 2016) and blasted against the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic Analysis. The phylogeny for each genus was reconstructed using the Maximum Likelihood (ML) method based on the Tamura-Nei model (Tamura and Nei, 1993). The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with a superior log likelihood value. Evolutionary analyses were conducted in MEGA 7 (Kumar et al., 2016).

Pathogenicity Testing. Spore suspensions were prepared by flooding PDA cultures with sterile distilled water amended with two drops of Tween 20 per 100 ml and the spore concentration was adjusted to 10^6 conidia ml^{-1} using replicate hemocytometer counts. For the pathogenicity test, sesame seeds were sown in a glasshouse under natural light conditions (14 h photoperiod, 25-32ºC). Ten sterilized seeds per pot were sown in a soil - sand - farmyard manure mixture (2:1:1 w/w). Plants, 4-weeks old, were sprayed to run off with the spore suspension using a spray bottle and covered with plastic bags to maintain high humidity for 24 h. Symptoms were evaluated 7 days after inoculation. Plants were rated for the presence (+) or absence (–) of 2- to 5-mm-diameter spots of irregular shape, dark brown to grey in color and surrounded by a bright yellow margin. Three replicate pots for each isolate were tested. To verify the ability of the *Alternaria* spp. isolates to colonize the

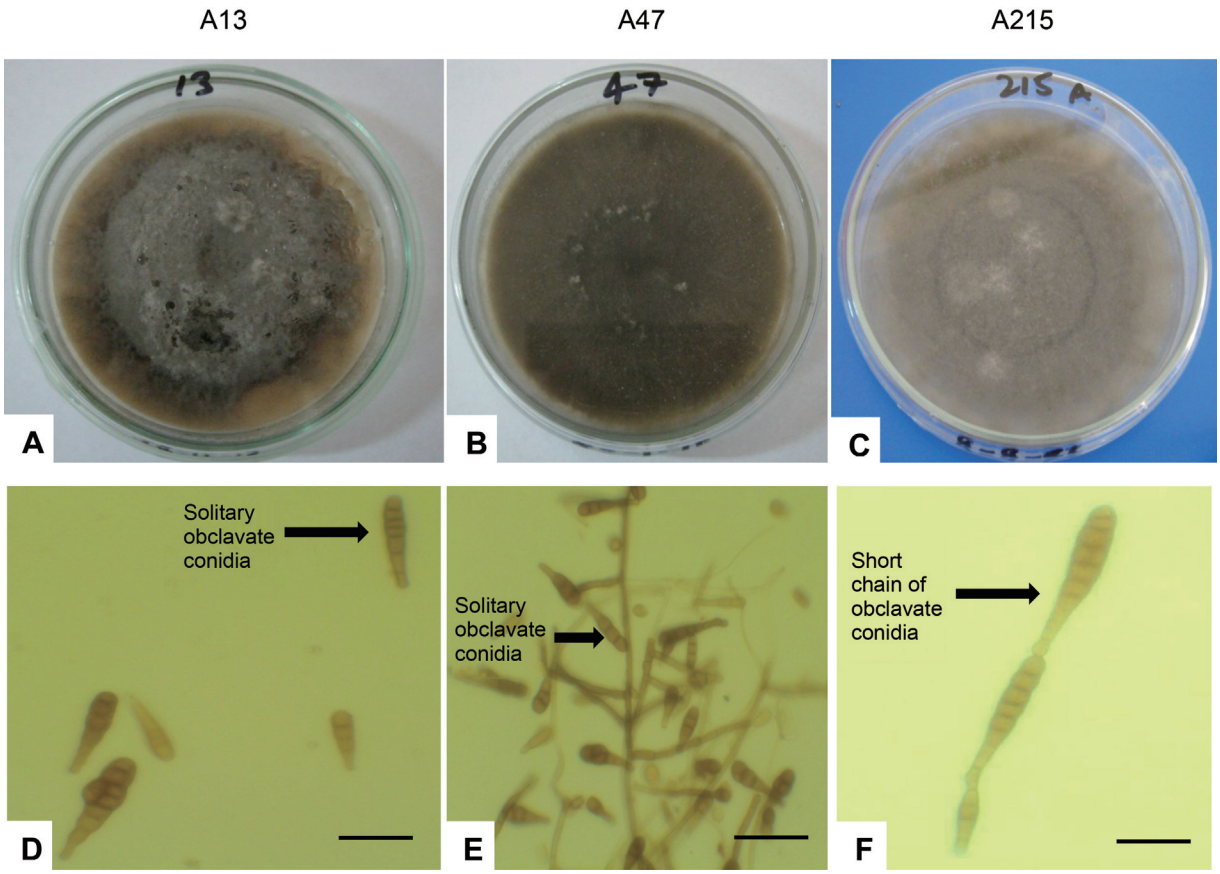

Fig. 2. (A-C) Colony morphology of three most frequent *Alternaria* isolates (A13, A47, A215) obtained from sesame seeds on PDA. (D-F) Conidia of three most frequent *Alternaria* isolates (Bar = 10 µm).

host tissues, re-isolations were made from the lesions onto PDA to fulfill Koch's postulates. In addition, disease severity (DS) was scored on a revised rating system from Zhao et al. (2016): 0 = no symptoms on leaves; 1 = 0-25% infection on leaves; 2 = 25-50% infection on leaf area. Disease severity index (DSI) was calculated as ∑ (disease severity scale points × number of plants at each scale point) / (total number of seedlings assessed × disease severity scale of the highest scale point observed) × 100 (Zhao et al., 2014).

Effect of Culture Filtrate on Seed Germination and Seedling Growth. Erlenmeyer flasks containing 50 ml of potato dextrose broth (Sigma Aldrich, USA; catalog number P-6685; prepared using 24 g/L and autoclaved at 121ºC for 15 minutes) were inoculated with the test fungi and incubated at 28ºC. After 5 days, the culture broth and fungal mycelia were carefully separated. A 50 ml volume of 3:2:1 (v/v/v) ethyl acetate: chloroform: methanol was added to each flask containing culture broth, followed by shaking overnight on a rotary shaker. Extracts were centrifuged at 5000 rpm for 30 minutes and the supernatant incubated in a water bath at 45ºC for 8-10 h to concentrate the extract to a volume of 10 ml (Jaiswal et al., 2012).

Sesame seeds were surface sterilized in 5% sodium hypochlorite for two minutes followed by 3 rinses in sterile distilled water before suspending in culture filtrates (10 ml). Following incubation at 28 ± 2ºC for 24 h, seeds were removed from the filtrate extract and washed once in sterile distilled water. Treated seeds were plated on 1.5% water agar in 90 mm diameter Petri dishes, with 10 seeds per dish. Control seeds were treated with distilled water prior to plating on 1.5% water agar. After 7 days of incubation, shoot and root lengths were recorded. In addition, a vigor index was calculated (Jalander and Gachande, 2012) following the formulae:

Germination % = Germinated seeds of treatment /Germinated seeds of control × 100

Vigor index = Seed germination (%) × Seedling Length (Shoot + Root Length)

Table 1. Morphological characterization, isolation frequency and relative density of *Alternaria* isolates from sesame seeds

Isolate code	Name of fungi	Origin (city)	Non sterilized seeds			Surface sterilized seeds		
			No. of isolates	*Fr*	*RD*	No. of isolates	*Fr*	*RD*
A6	*Alternaria dianthi*	Sialkot	5	10	2.14	4	6	2.06
A13	*Alternaria sesami*	Sialkot	58	24	24.79	32	40	16.49
A19	*Alternaria citri*	Sialkot	13	18	5.56	5	8	2.58
A47	*Alternaria longipes*	Gujranwala	17	78	7.26	19	40	9.79
A91	*Alternaria dianthicola*	Gujranwala	17	24	7.26	7	10	3.61
A166	*Alternaria brassicicola*	Gujranwala	6	8	2.56	32	40	16.49
A181	*Alternaria solani*	Gujranwala	7	10	2.99	3	2	1.55
A183	*Alternaria raphanin*	Gujranwala	0	0	0.00	1	2	0.52
A196	*Alternaria alternata*	Gujranwala	13	18	5.56	5	6	2.58
A203	*Alternaria dianthicola*	Hafizabad	16	22	6.84	4	6	2.06
A215	*Alternaria brassicae*	Hafizabad	18	26	7.69	30	26	15.46
A217	*Alternaria citri*	Hafizabad	2	2	0.85	0	0	0.00
A218	*Alternaria infectoria*	Hafizabad	3	4	1.28	0	0	0.00
A220	*Alternaria sesamicola*	Gujrat	17	24	7.26	6	8	3.09
A221	*Alternaria helianthi*	Gujrat	9	12	3.85	6	8	3.09
A228	*Alternaria longissimi*	Gujrat	10	14	4.27	3	4	1.55
A236	*Alternaria raphanin*	Gujrat	4	4	1.71	1	2	0.52
A239	*Alternaria tenuissima*	Attock	7	10	2.99	0	0	0.00
A249	*Alternaria triticina*	Attock	0	0	0.00	19	26	9.79
A261	*Alternaria radicina*	Mandi Bahuddin	4	4	1.71	4	8	2.06
A263	*Alternaria pluriseptata*	Mandi Bahuddin	3	6	1.28	2	2	1.03
A267	*Alternaria cinerariae*	Mandi Bahuddin	2	4	0.85	5	8	2.58
A272	*Alternaria chlamydospora*	Mandi Bahuddin	3	4	1.28	6	6	3.09

Fr = Isolation frequency; *RD* = relative density.

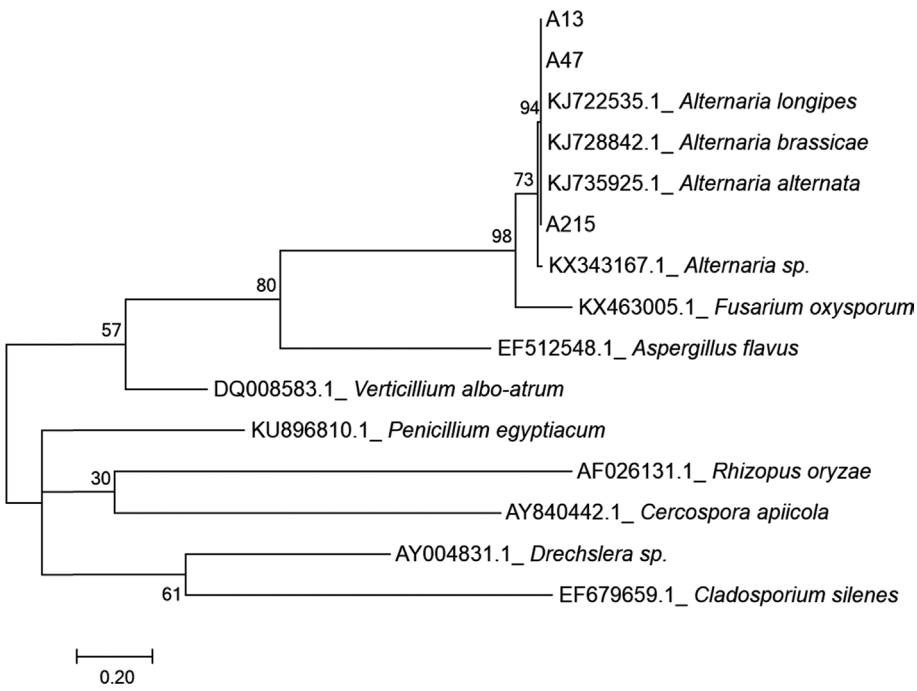

Fig. 3. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Alternaria* isolates from sesame seeds using ITS rDNA primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.

Results

Morphological Identification of *Alternaria* species. A total of 428 isolates of *Alternaria* were recovered from sesame seeds collected in the Punjab, Pakistan. Seeds were selected from plants exhibiting blight symptoms on leaves (Fig. 1B) and pods (Fig. 1C). These isolates were cultured to homogeneity: all isolates developed loose, cottony and greyish-green to olive brown colonies on PDA after incubation at 25ºC for 7 days in the dark (Fig. 2A, B, C). Isolates were grouped morphologically, based on well-established features of *Alternaria* species, focusing on colony characteristics and conidial structure (Fig. 2). Based on these features, the isolates were placed into 36 morphological groups and identified as far as possible. Isolates produced conidia as solitary or in short chains. Conidia were narrow-obclavate, ovoid or ellipsoid with 1-2 longi-septa.

The mean isolation frequency and relative density of each isolate form was determined in 100 non-surface sterilized and in 100 surface sterilized seeds. It was observed that majority of isolates were obtained from Gujranwala followed by Hafizabad, Gujrat and Mandi Bahuddin. Quantitatively three isolate groups (A13, A47, A215) were found most frequently (Table 1) and selected for further analyses.

Identification by DNA sequencing. PCR amplification of the ITS regions of rDNA yielded fragments of ~550 bp from all tested *Alternaria* isolates. PCR of the *Alt a 1* gene gave fragments of ~500 bp in length from all isolates. BLAST searches on NCBI showed the ITS sequences to be 100% identical to those of *Alternaria* isolates described as "*Alternaria* sp." but also matched precisely with named species including *A. alternata*, *A. longipes* and *A. brassicae*. Phylogenetic analysis using ITS sequences failed to differentiate the isolates, grouping all 3 into a single clade (Fig. 3), with the reference sequences of *A. alternata* (KJ735925.1), *A. brassicae* (KJ728842.1) and *A. longipes* (KJ722535.1) obtained from GenBank. In contrast, sequences of the *Alt a 1* gene showed > 99% homology with *Alternaria alternata* and phylogenetic analysis placed the isolates in a clade close to the reference sequence of *Alternaria alternata* (KP123850.1; Fig. 4). Similarly, some morphological characters of selected isolates (A13, A47 and A215) showed homology with colony and conidial characters of *A. alternata.* However, the *Alt a 1* sequence of strain A13 indicated that it was distinct from the *A. alternata* clade. Sequences from the isolates obtained in this work were submitted to GenBank (Table 2).

Pathogenicity Tests. Seven days after inoculation with a spore suspension of the *Alternaria* isolates, brown lesions surrounded by yellow haloes began to develop on sesame leaves; disease symptoms gradually spread from leaf margins to the midveins, similar to symptoms observed in the sesame fields during the sample collection. In a later stage, blight extended to the center of the leaves and plants be-

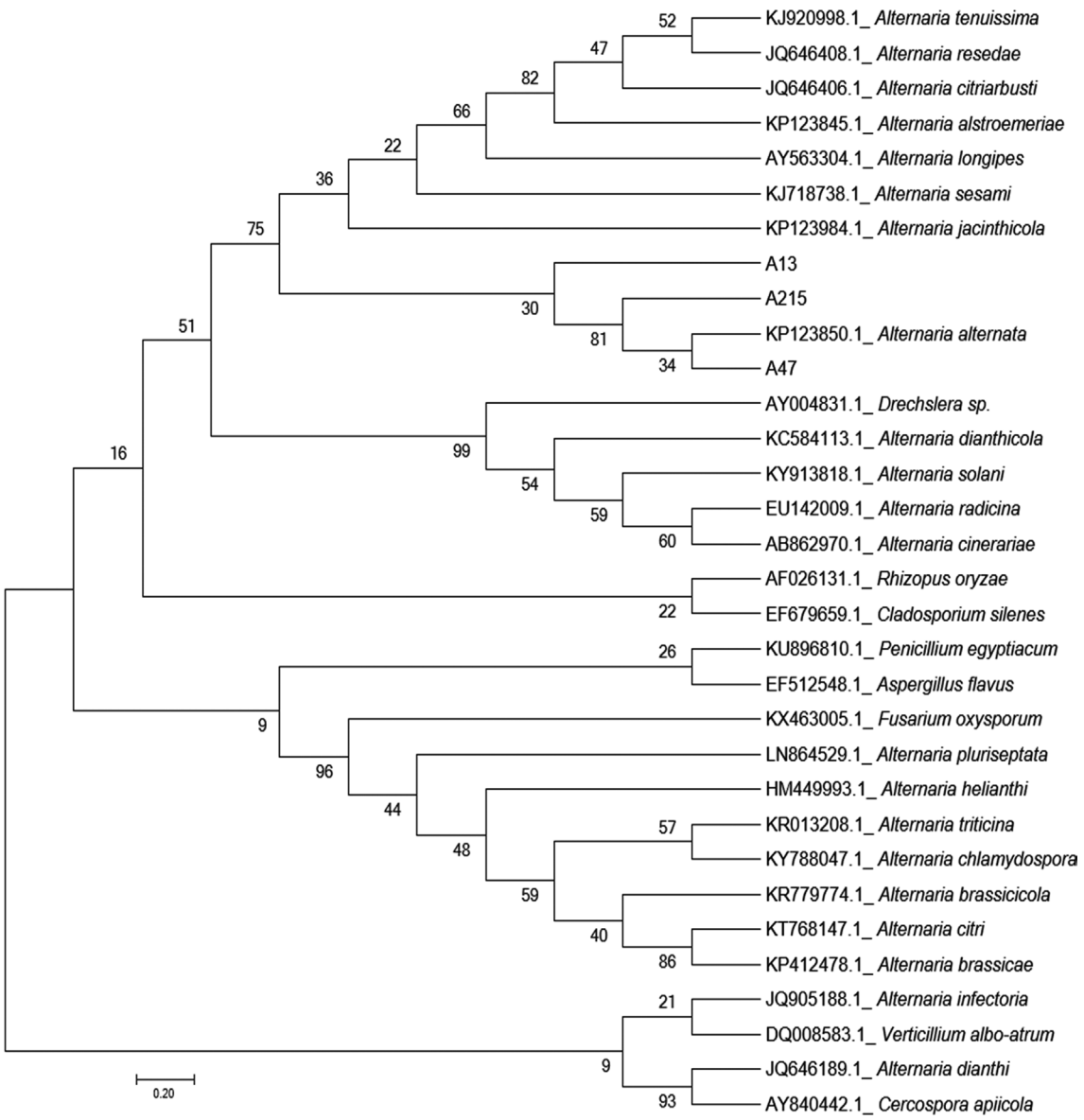

Fig. 4. Maximum likelihood phylogenetic tree obtained from consensus sequences of *Alternaria* isolates from sesame seeds using Alt-forward and reverse primers. Numbers above the branches are bootstrap values from 500 replicates. The scale indicates the genetic distance between the species.

Table 2. Molecular Identification of three most frequent *Alternaria* isolates obtained from sesame seeds

Isolate code	Origin (city)	Morphological identification	Molecular identification		NCBI accession no.	
			ITS	*Alt a 1*	ITS	*Alt a 1*
A13	Sialkot	*Alternaria sesami*	*Alternaria* sp.	*Alternaria alternata*	KY190101	KY124234
A47	Gujranwala	*Alternaria longipes*	*Alternaria* sp.	*Alternaria alternata*	KY190102	KY124235
A215	Hafizabad	*Alternaria brassicae*	*Alternaria* sp.	*Alternaria alternata*	KY190103	KY124236

came defoliated (Fig. 5). Extended necrosis surrounded by yellowing was usually observed on diseased leaves. Pathogenicity tests confirmed that all tested isolates were virulent leaf blight pathogens, the most virulent being A13 (Table 3). No symptoms developed on control plants. The *Alternaria* isolates were reisolated from symptomatic leaves of inocu-

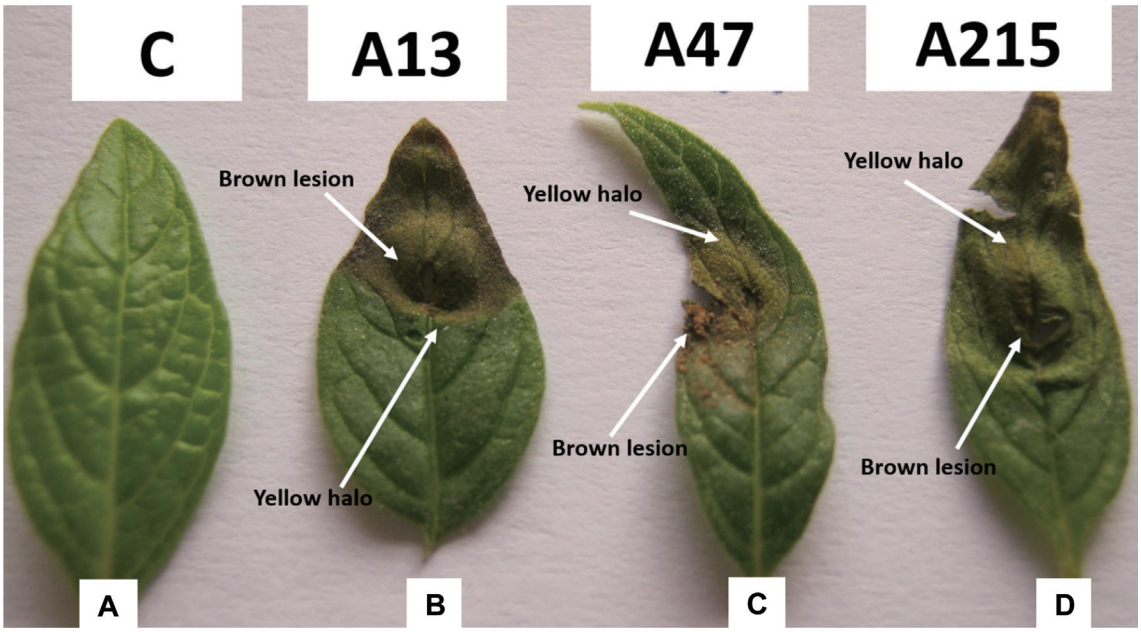

Fig. 5. Symptoms produced by three isolates of *Alternaria* on leaves of sesame plants 7 days after inoculation. (A) Control; (B) A13; (C) A47; (D) A215.

Table 3. Pathogenicity test of *Alternaria* isolates (A13, A47 and A215) on sesame seedlings

Infection	Total leaves	Infected leaves	Disease incidence (%)	Disease severity incidence	No. of lesions/leaf	Area of lesions (cm)	Area of leaf	Infection (%)	Rating scale
A13	7	2	28.5	28.57	2	0.49	1.55	31.61	2
A47	9	2	22.2	12.11	2	0.22	1.16	18.97	1
A215	10	1	10	20	1	0.5	1.19	42.02	2

Fig. 6. Effect of cultural filtrates of *Alternaria* isolates on the germination of sesame seeds after 7 days of incubation. (A) Control; (B) A13; (C) A47; (D) A215.

lated plants, but not from any of the control plants.

Effect of Culture Filtrates on Seed Germination. Seed germination and vigor of sesame seedlings were adversely affected by the culture filtrates of isolate A13 (Fig. 6). The reduction in seed germination ranged from 40-60%, while vigor index in samples treated with filtrates of this isolate was 4% compared with over 62% in control plants (Table 4).

Discussion

This work clearly demonstrated that *Alternaria* species are present in a high proportion of sesame seeds collected from various regions in the Punjab, Pakistan, suggesting that there is a potential source of inoculum to initiate disease outbreaks under conditions favorable to pathogen develop-

Table 4. Effects of culture filtrates from *Alternaria* isolates (A13, A47 and A215) on germination of sesame seeds and growth of seedings

Treatment	Germination %	Root length (cm)	Shoot length (cm)	Vigor index
A13	40.00	0.10	0.00	4.00
A47	60.00	0.40	0.90	24.90
A215	53.33	0.20	0.00	10.67
Control	86.67	0.70	1.50	62.17

Vigor index = Seed germination (%) × Seedling Length (Shoot + Root Length).

ment. The most common species of *Alternaria* found in the present work was *A. alternata*. Species delineation within the genus *Alternaria* requires careful attention in order to determine the range of species causing diseases on a given host.

Amongst the species of *Alternaria* identified morphologically, three representatives, chosen from the most commonly occurring groups (A13, A47, A215), were initially identified as *Alternaria sesami*, *Alternaria longipes* and *Alternaria brassicae*, respectively, on the basis of conidial morphology and colony characteristics. Identification of *Alternaria* species based on morphological criteria, however, remains a challenging task, leading to controversy and confusion very often accompanying classification in this genus (Maraite et al., 1998). This problem is especially relevant for the small-spored species, which share morphological characteristics such as having overlapping conidial size ranges (Simmons, 1992). Although the ITS region of rDNA is widely used as a barcode for fungi (Blaalid et al., 2013), the sequences of the *Alternaria* isolates analyzed here were 100% identical to those of "*Alternaria* sp." in Genbank, but were also identical to those of other *Alternaria* species, including *A. alternata*, *A. longipes* or *A. brassicae*. It has been recognized that ITS sequences are not always able to differentiate at the species level in the genus *Alternaria* (Pryor and Michailides, 2002; Shipunov et al., 2008; Zur et al., 2002).

In contrast, use of the partial coding sequence of the *Alternaria* major allergen gene (*Alt a 1* gene) in PCR enabled accurate identification to the species level, confirming that all three isolates tested were *Alternaria alternata*, in agreement with other workers studying different hosts for this genus of pathogens (Guo-yin et al., 2013; Paul et al., 2015; Skóra et al., 2015). It has been suggested that the *Alt a 1* gene sequence evolved 3.8 times faster and contained 3.5 times more parsimony-informative sites than glyceraldehyde-3-phosphate dehydrogenase (*gpd*) sequences, strongly supporting the use of this part of the genome for species identification in *Alternaria* (Hong et al., 2005a).

Based on this work, therefore, it can be concluded that *Alternaria alternata* is a destructive pathogen causing leaf blight on sesame in the Punjab, Pakistan. The incidence of *Alternaria alternata* in sesame seed was high compared to other species of *Alternaria* isolated from this host plant. Most *Alternaria* spp., including *A. alternata*, exhibit considerable morphological plasticity depending on cultural conditions, including substrate, temperature, light and humidity. These fungi, therefore, in particular *A. alternata*, can be frequently misidentified when relying on morphological characteristics alone (Misaghi et al., 1978; Roberts et al., 2000; Simmons, 1992). Moreover, the inoculation tests carried out with the three isolates of *Alternaria alternata* confirmed pathogenicity and virulence, with the development of leaf blight in sesame, similar to the results reported with *A. longipes* by Shoaib et al (2014).

The wide variability among different *Alternaria* species from different hosts has been reported previously (Kumar et al., 2008; Pryor and Gilbertson 2000; Pryor and Michailides, 2002; Quayyum et al., 2005). Among the different diseases caused by species in the genus *Alternaria*, leaf blight disease is one of the most important, causing yield loss in the range of 32-57% per annum in canola, for example (Conn and Tewari, 1990). In the present study, symptoms of the disease on sesame include the presence of irregular, often circular brown to dark brown colored spots on the leaves, with concentric lines inside the spots which are in agreement with the findings of Valkonen and Koponen (1990) who reported that individual circular spots may coalesce to form large patches, resulting in leaf blight of Chinese cabbage. In some infections, small dark colored spots are formed on pods and young shoots of Chinese cabbage.

An important conclusion drawn from the work presented in this paper is that identification of *Alternaria* spp. on the basis of morphological characteristics alone is unreliable for correct determination of species. More stringent identification was possible using species-specific primers, which are required in diagnostics for sesame seed testing: ITS sequences were not sufficiently discriminatory to separate species.

Acknowledgments

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