Morphometric and internally transcribed spacer gene sequence-based characterization of *Alternaria burnsii*

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***Alternaria burnsii*, the pathogen responsible for cumin blight, was collected from diseased plant samples from North Gujarat. AB-01 showed maximum growth at 28 ± 1°C. Conidia ranged from 44.92 to 63.28 µm in length and 10.84 to 24.36 µm in width whereas beak length of conidia ranged from 20.34 to 47.85 µm. The isolate AB-01 showed the highest sporulation frequency (1.24×10^5/ml), but the highest percentage of disease intensity was observed in AB-08 (31.4). Internally transcribed spacer gene sequence based phylogenetic grouping using MEGA5.6 and the factorial analysis using DARwin5 reflect the presence of two distinct groups. The fast growing isolates that show high pathogenicity are present in group-I of the dendrogram whereas the members of group-II show grey black colony colour, light brown colony margin, plain irregular growth pattern, and comparatively larger beak length of conidia.**

Keywords: *Alternaria burnsii*, blight, conidia, ITS, MEGA.

INDIA is the largest producer (area 0.8 mi ha, production 460 thousand tonnes) and consumer of cumin (*Cuminum cyminum* L.) seed in the world and contributes about 73% to the global production[1]. In India, cumin seed is mainly produced in the states of Gujarat and Rajasthan; Gujarat is the largest producer with 86.5% market share[2]. Despite good potentiality of qualitative and quantitative improvement in cumin seed production in this region, a wide gap exists between the potential and actual yield of the crop. Among various factors affecting cumin yield, blight disease caused by the fungus *Alternaria burnsii* is one of the most predominant diseases, which causes considerable qualitative and quantitative yield losses. It results in a disease severity of 16–65% and under favourable climatic condition the pathogen causes severe damage[3] and even almost complete failure to the cumin crop.

Blight is one of the most destructive diseases in cumin caused by the fungus *Alternaria burnsii*. This pathogen survives in crop debris and is transmitted through seeds. The disease development is favoured by cool, humid and persistent cloudy weather. Moist weather and wind help the pathogen spread fast; and eventually the infested field turns brownish in patches. The disease is characterized by the development of small, isolated, whitish necrotic areas on the aerial plant parts, which gradually enlarge and coalesce with each other, turning purple, eventually brown and finally black. In severe cases, the infection readily spreads to the stem and blossom and kills the succulent leaves and blossoms and there may be no seed production. Even if seeds are produced, they are shrivelled, dark-coloured, light, and are usually non-viable.

Identification and classification of *Alternaria* species had been mainly based on morphological characters such as colour, size, shape of conidia and conidiation patterns as well as pigmentation. On this basis, *Alternaria* has been placed into fourteen distinct morphological species-groups[4]. The agent of blight disease of cumin (*Alternaria burnsii*) also varies widely in its morphology and cultural characters. However, a detailed study encompassing a reliable molecular tool for characterizing this fungus along with pathological, cultural and morphological attributes is lacking.

Recently, molecular approaches have increasingly been used in taxonomy and systematics of filamentous fungi including phytopathogens at the species and sub-species level[5]. However, very few studies have focused on the characterization and diversity of *Alternaria burnsii*. Moreover, such studies have selected few isolates and the findings thereof are not supported by gene sequence information. Sequence-based information especially internally transcribed spacer (ITS) gene sequences are considered powerful molecular tools for phylogenetic analysis and are even able to discriminate among species that are identical based on biochemical and morphological characters[6]. The ITS gene sequence has been useful in classification of fungi[7] and in systematic and taxonomic studies due to its suitable size for PCR amplification, restriction analysis and sequencing procedures, and also because ITS regions are variable among species[8]. With this background, the present study was undertaken to differentiate the plant pathogenic isolates of *Alternaria burnsii*, obtained from the blighted cumin plants, using morphometric and ITS gene sequence. This study scores over others in being the first ever ITS gene sequence-based study of *Alternaria burnsii*, which is also supported by morphological and conidial characteristics.

Blighted cumin plants showing typical blight symptoms were collected randomly from the cumin growing belt of north Gujarat. The pathogen responsible for blight was isolated from the diseased aerial plant parts (leaves, stem, branches, pedicels and seeds) by standard tissue isolation technique[9]. Pure culture of the pathogen prepared by single spore isolation under microscope was maintained on slants containing potato dextrose agar medium and was identified following cultural and morphological characteristics and the keys given by

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Alexopoulos[10]. Thus, ten isolates of the fungus *Alternaria burnsii* were obtained and named sequentially from AB-01 to AB-10. These isolates were tested for pathogenicity on *Cuminum cyminum* (cultivar GC4) and the disease symptom and its causal agent were authenticated. Per cent Disease Intensity (PDI) was recorded on 0–5 scale (0 = healthy plants; 1 = blight symptoms on tips of leaves; 2 = most leaves showing blight symptoms; 3 = symptoms on leaves and umbel; 4 = symptoms on leaves, umbel and few lesions on the stem; and 5 = symptoms on leaves, umbel, seed and on the stem) following the formula, PDI = sum of rating scales of observed plants/total number of observed plants × 1/ maximum rating scale × 100.

The cultural variability of the isolates was studied by growing them on PDA medium. This was done by inoculating the centre of the pre-poured petri plates having PDA medium with a 5 mm disc of ten days old actively growing pure culture and incubating the plates at 28 ± 1°C in a BOD incubator in five replications. The colony characters, pigmentation and growth habits were visually observed after each 24 h of incubation, until one week of incubation. Sporulation intensity was calculated by placing and washing a 5 mm disc of 2 weeks old culture in 2 ml of sterilized distilled water with camel hair brush followed by a second washing in 3 ml of water. The composite 5 ml of fungal spore suspension was used for counting total conidia/ml with the help of haemocytometer[11].

The isolates were studied for their morphological characters by staining ten-day-old culture with lactophenol–cotton blue. Observations on the size of conidia, and the number of transverse and longitudinal septa in conidia were recorded using a compound light microscope (Nikon, Japan) and the images analysed using the NIS-Elements Documentation software (Nikon, Japan). Randomly, fifty conidia from each slide were examined at 40× magnification of the light microscope and measured using the software. The average was used to calculate the conidial length, width and number of transverse and longitudinal septa.

Fungi genomic DNA was isolated from the culture using Genomic DNA isolation kit (Merck, India) according to the manufacturer protocol. The isolated DNA samples were treated with 100 µl of RNAase A (10 mg/ml) (GeNei, India) at 37°C for one hour to remove RNA contaminants. The quantity and quality of genomic DNA were estimated using UV spectrophotometer (Metertech Inc., Taiwan) and the DNA samples diluted to a concentration of 20 ng/µl.

The internally transcribed spacer sequence of approximately 1200 bp between the 18S and 28S rRNA gene was amplified using high-fidelity PCR polymerase and universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′)[12]. The polymerase chain reaction was carried out in a final volume of 50 µl having 1× assay buffer 5A, 2.5 mM each of dATP, dCTP, dGTP and dTTP, forward and reverse primers in equimolar ratio (100 ng each), 3U of XT-5 DNA polymerase and 20 ng of genomic DNA. Amplification was achieved in a fast thermal cycler (LongGene, Zhejiang, China) programmed for initial denaturation (94°C for 5 min) followed by 30 cycles of denaturation (94°C for 30 sec), annealing (56°C for 30 sec), extension (72°C for 1 min 30 sec) and a final extension of 10 min at 72°C and subsequent cooling at 4°C.

Amplification products were separated along with StepUp 500 bp DNA ladder (Genei, India) by electrophoresis on 1.0% agarose (Genei, India) gel (stained with 1% ethidium bromide) run in 1XTAE buffer for a period of 2.5 h at 75 volts. The bands were visualized under UV light and gel photographs were scanned through FireReader gel documentation system (UVItec Limited, UK) and the amplification product sizes were evaluated using software FireReader V4 1D (UVItec Limited, UK).

The PCR products/bands were cut and purified using QIAquick Gel Extraction Kit (Qiagen) and gene sequencing was performed using Big Dye terminator cycle sequencing kit (Applied BioSystems, USA). Sequencing products were resolved on an Applied Biosystems model 3500XL automated DNA sequencing system (Applied BioSystems, USA). These sequences were compared for similarity with sequences present in NCBI (National Centre for Biotechnology Information, NIH, USA) database. The sequences from all isolates were aligned using ClustalW alignment algorithm and the evolutionary history was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method and the evolutionary distances were computed using maximum composite likelihood method using MEGA5.6 (ref. 13). The principal coordinate analysis (factorial analysis) for further grouping of the *A. burnsii* isolates was done using the DARwin5 software[14].

The ten isolates of *Alternaria burnsii* obtained from diseased plants varied widely in cultural characteristics on the potato dextrose agar medium (Figure 1). The pure culture of pathogen varied widely in appearance and showed whitish brown, brownish black, greenish dark black, grey black and dark black colonies whereas the colony margin was dirty white, light brown, brownish and blackish in colour and the growth pattern observed was plain or fluffy, regular or irregular radial growth and sometimes knotting (Table 1). The isolate AB-01 (50.5 mm) showed maximum growth on day 4 of incubation followed by AB-03 (42.2 mm) and AB-10 (41.5 mm). However, on day 7 of incubation the isolate AB-01 (76.5 mm) again showed maximum growth followed by the isolates AB-04 (69.5 mm) and AB-03 (67.5 mm).

A. burnsii revealed the presence of branched, erect, straight, irregularly bent and geniculate conidiophores. The conidia produced by these isolates varied widely in

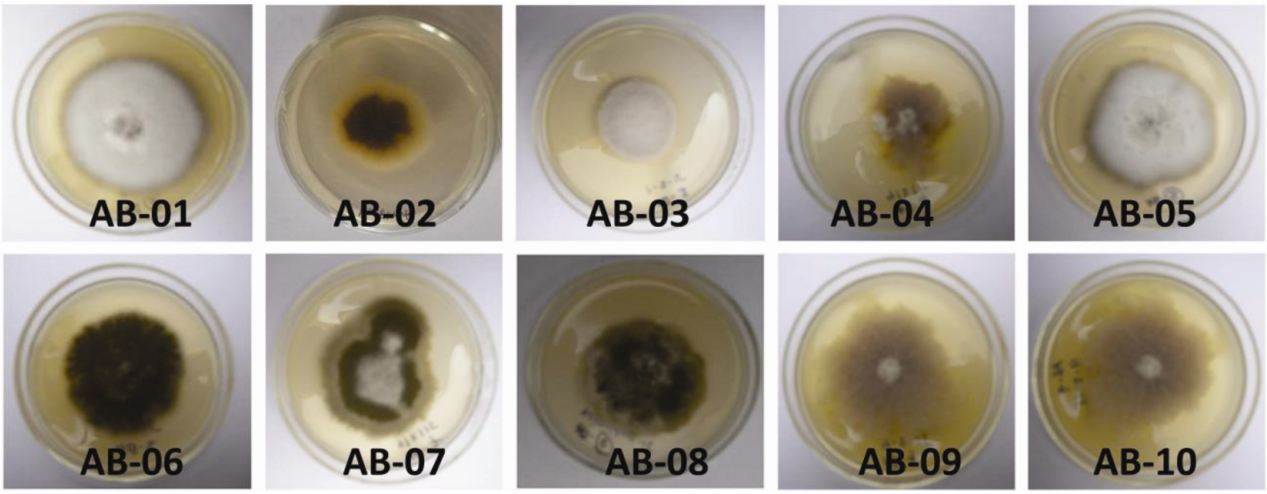

Figure 1. Growth of isolates of *Alternaria burnsii* on potato dextrose agar medium.

their size, number of longitudinal or transverse septa, beak length and sporulation frequency. The size of conidia ranged from 44.92 to 63.28 µm in length whereas the width ranged from 10.84 to 24.36 µm. This group of fungus is known to invariably produce beaked conidia and varied widely in the number of longitudinal and transverse septa. The beak size of conidia ranged from 20.34 to 47.85 µm whereas the number of longitudinal septa varied from 0–1 to 0–2 and the transverse septa from 0–3 to 1–5. The isolate AB-01 showed the highest sporulation frequency (1.24×10^5/ml); however the highest percentage of disease intensity was observed with the isolate AB-08 (31.4) (Table 1).

Individual isolates of *A. burnsii* have been observed to produce more than one type of conidia. However, a close scrutiny in this aspect led us to categorize various conidia produced by different isolates into five groups, a – conidia with only transverse septa and no beak, b – conidia with both transverse and longitudinal septa and no beak, c – conidia with only transverse septa and beak, d – conidia with both transverse and longitudinal septa and short (<15 µm) and medium beak (15–30 µm), and e – conidia with both transverse and longitudinal septa and long beak (>30 µm) (Figure 2).

Amplification of internally transcribed spacer gene sequences of fungal isolates yielded a specific band of approximately 1200 bp. The ITS gene sequences of isolates were matched for similarity using BLAST and were submitted in the NCBI GenBank with accession numbers KR604836, KR604837, KR604838, KR604839, KR604840, KR604841, KR604842, KR604843, KR604844 and KR604845 sequentially for the isolates AB-01 to AB-10. Multiple sequence alignment using ClustalW alignment algorithm reflected the diversity and variation present among the isolates. This alignment reflects multiple addition, deletion and substitution in the nucleotide sequence of the isolates. The phylogenetic tree prepared using maximum composite likelihood method using MEGA5.6 placed these isolates into two well distinct groups. The first group in the circular phylogenetic tree was represented by the seven isolates other than the isolates AB-04, AB-09 and AB-10 (represented the second group) (Figure 3). The bootstrap values were in the range of 46–100, which provided high degree of confidence to the identification and phylogenetic classification of these fungal isolates.

The principal coordinate analysis (Axes 1/1) of the *A. burnsii* ITS gene sequences based on dissimilarity matrix grouped these isolates again into two groups, which varied widely in their genetic characteristics (Figure 4). The principal/factorial coordinate analysis showed a result analogous to that of clustering pattern. The members of the group encircled by a solid line comprised seven isolates (group-I) which showed an average similarity coefficient of more than 0.98 whereas the members of the second group (encircled by a dotted line) comprised three isolates (group-II) with a similarity of about 0.99. From this finding, it was quite evident that the grouping of isolates in the coordinate analysis was in congruence with that of the findings of the phylogenetic tree.

Cumin (*Cuminum cyminum* L.) is a highly remunerative cash crop of NW semi arid region of India. Plant pathogenic fungi belonging to the genus *Alternaria* infect a wide range of host plants and are major causes of agricultural yield losses[15]. Uppal *et al.*[3] first reported *Alternaria* blight of cumin from Gujarat (India), which has now become one of the most common diseases all over the cumin-growing areas. The plants are normally infected by the pathogen only during or after the flowering stage and the disease is favoured by humid and cloudy weather and results in the development of small necrotic spots all over the aerial plant parts which enlarge, coalesce and turn brown to black. Under severe infestation, the stem and flowers are also infected and may die. Either

Table 1. Cultural and conidial characteristics of the cumin blight pathogen

	Cultural characteristics					Conidial characteristics					
		Colony growth (mm)		Colour of colony		Conidia size	Beak	Septa		Sporulation	Per cent disease
Strains	Colony colour	Day 4	Day 7	margin	Growth pattern	($L \times B$) (µm)	length (µm)	Longitudinal	Transverse	(ml)	intensity
AB-01	Whitish brown	50.5	76.5	Brownish	Fluffy radial	47.48 × 10.84	20.34	0–1	0–3	1.24×10^5	19.5
AB-02	Dark black	27.5	43.2	Dirty white	Fluffy radial	55.18 × 22.28	28.37	0–2	1–3	9.15×10^4	14.1
AB-03	Whitish brown	42.2	67.5	Dirty white	Fluffy radial	44.92 × 15.28	24.57	0–1	1–3	1.18×10^5	15.8
AB-04	Grey black	40.4	69.5	Light brown	Plain irregular knotting	51.98 × 19.70	41.01	0–1	1-4	1.04×10^5	17.8
AB-05	Dark black	32.5	45.5	Brownish	Fluffy irregular radial	63.28 × 21.28	27.91	0–1	1–5	6.75×10^4	29.0
AB-06	Brownish black	37.4	56.5	Blackish	Fluffy irregular radial	56.52 × 24.36	32.64	0–2	1–4	8.34×10^4	12.7
AB-07	Greenish dark black	37.2	48.5	Brownish	Fluffy knotting	58.90 × 22.82	32.68	0–2	0–5	7.64×10^4	23.2
AB-08	Dark black	30.5	46.5	Brownish	Fluffy radial	57.38 × 20.78	36.87	0–2	1–5	6.25×10^4	31.4
AB-09	Grey black	37.2	42.8	Light brown	Plain irregular radial	60.80 × 23.00	47.85	0–2	1–5	8.33×10^4	21.5
AB-10	Grey black	41.5	59.5	Light brown	Plain irregular radial	61.60 × 20.16	42.55	0–2	1–5	8.14×10^4	25.7
SEm ±		0.78	0.91								0.36
CD (0.050)		2.20	2.59								1.02
CV (%)		5.86	3.34								3.01

Values for spore size, beak size and number of longitudinal and transverse septa indicate average of 50 observations.

Figure 2. Conidial morphology of the fungus *Alternaria burnsii*. ***a***, Conidia possess only transverse septa and no beak; ***b***, Conidia possess both transverse and longitudinal septa and no beak; ***c***, Conidia possess only transverse septa and beak; ***d***, Conidia possess both transverse and longitudinal septa and short (<15 µm) and medium beak (15–30 µm); ***e***, Conidia possess both transverse and longitudinal septa and long beak (>30 µm).

seeds are not formed on diseased plants or, if produced, they remain small, shrivelled, very light and blackish in colour[16,17].

The disease development is favoured by cool, humid, persistent cloudy weather and temperature from 11.34 to 28.07°C (ref. 16). Several studies have tested chemical fungicide, botanicals and biological agents for control of diseases caused by *Alternaria*. The survivability of this fungus on seeds remains 100% during April–May but it reduces to 70% in October–November. However, its survivability in crop debris remains in the range of 40–60%[18]. Early planting results in greater severity of the disease. The pathogen is seed-borne and seed-transmitted. It is also soil-borne surviving in crop debris. The volatile oil content of seeds is reduced due to the disease. The incidence of *Alternaria* blight increased with increase in the duration of dewfall, leaf wetness, relative humidity, congenial temperature and number of rainy days[19].

Cultural variability in *A. burnsii* has been reported by Pipaliya and Jadeja[20] but it has not been supported by extensive data and genetic information. Considerable variations in cultural and morphological characteristics have also been reported[21]. They observed a pathogenic variability (PDI) of 24.2–65.4 when inoculated on pot-grown plants of cumin. They further observed significant variations in symptoms and latent period by the isolates on blight disease development. Sharma and Pandey[22] observed biochemical variability in protein profile. The virulent isolates of pathogen show a larger number of protein bands with high molecular weight, are fast growing, and cause maximum disease incidence when compared to isolates having fewer protein bands. The larger number of protein bands is probably associated with the presence of more enzymes responsible for pathogenicity[23].

The multiple sequence alignment of the internally transcribed spacer gene sequence indicated a distinct dichotomy among the isolates of *A. burnsii*. The ITS gene sequence groups these isolates into two groups and indicates existence of wide variability among them. This may be due to transfer of genes between and among distantly related species of fungus when they survive on crop debris or when host plants are not present in the fields, but climatic conditions are favourable. The members of the second group show grey-light brown colony colour, produce comparatively long beaked conidia, may or may not produce longitudinal septa and essentially produce transverse septa in conidia, and possess considerable sporulation intensity. Hence, it may be an indication of further speciation or development of new pathotype in this species of plant pathogen, which may be at an intermediate stage of evolution.

Although morphological and cultural characteristics of isolates of this fungus indicate high variability, they offer good potential to use molecular characterization, especially sequence based characterization of isolates, to

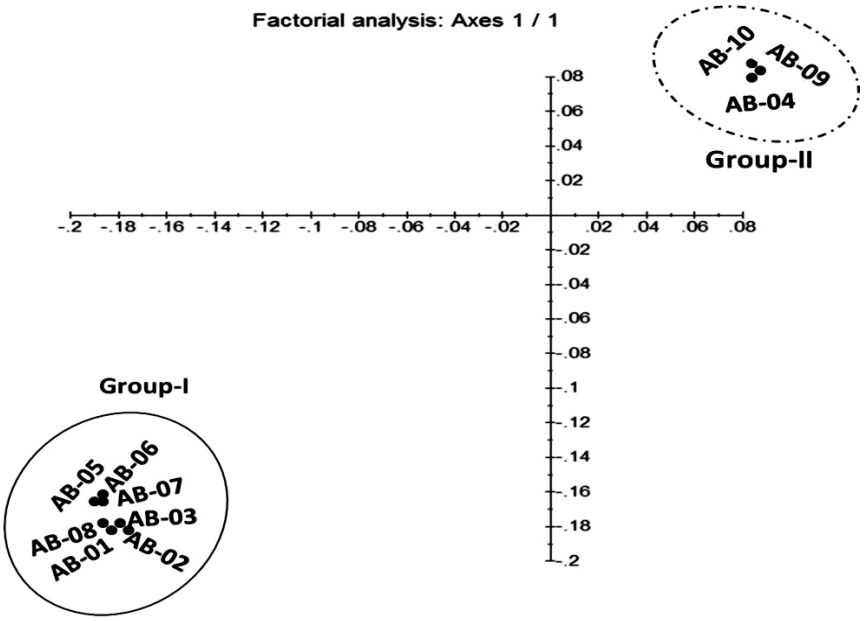

Figure 4. Principal coordinate analysis of the *A. burnsii* isolates using DARwin5.

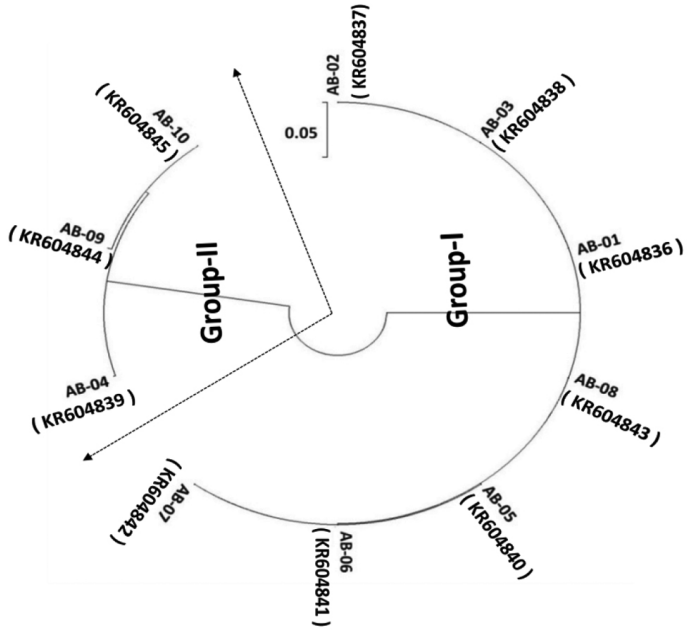

Figure 3. Phylogenetic grouping of *A. burnsii* isolates using MEGA5.6. GenBank accession number of the isolates in parentheses.

decipher their evolutionary characteristics and to differentiate them from other fungus or less pathogenic or saprophytic isolates of the same species. The present study reflects a positive correlation between the cultural characters like, grey-black colony colour, light brown margin, plain irregular growth pattern, comparatively larger beak length of conidia with the molecular diversity of the isolates. The isolates showing these morphological and cultural characteristics are grouped together in the same branch of the dendrogram (group-II) obtained with ITS gene sequences. Moreover, the isolates that grow fast and show high pathogenicity cluster together in group-I of the dendrogram. The variation in the cultural, morphological, and genetic attributes of the group-II indicates existence of horizontal gene transfer (HGT) among various species of fungus existing in the agroecosystems. This leads to stable integration of genetic material following transfer between individuals and excludes transfer through

meiotic or mitotic processes[24]. Moreover, several plant-pathogenic *Alternaria* species are known to carry conditionally dispensable chromosomes (CDCs) which may be transmitted horizontally between isolates in a population, potentially conferring new pathogenic attributes to the receiving isolate[25]. Loss of CDC can also occur during repeated subculturing, resulting in the transition from a pathogenic to saprophytic form of the fungus[26]. Such events of gene transfer are exemplified by nucleotide sequence similarity and structural comparisons between genes from members of both groups. Moreover, highly identical supernumerary chromosomes in *Alternaria* strains indicate their transfer into nonpathogenic ones by horizontal gene transfer which provides a possible mechanism by which pathogens with novel host specificities might have arisen in nature.

The isolates of *A. burnsii* varied widely in cultural, morphological and conidial characteristics. Although these attributes are valuable in identifying the plant parasitic pathogen, they do not give a deep insight into the evolutionary relationships and genetic variation among the isolates. However, the molecular characterization on the basis of internally transcribed spacer gene sequence differentiated these plant parasitic fungal isolates into two distinct groups. The factorial coordinate analysis also endorsed the phylogenetic grouping and differentiated the isolates into two groups, and deciphered the differences between them that were very clear in the morphological and cultural characteristics. This may be an indication of further speciation or development of new pathotype in this species of plant pathogen, which may be at an intermediate stage of evolution.

1. Indianspices.com.http://www.indianspices.com/pdf/Major%20-spice%20state%20wise%20 area% 20 production-2011-12.pdf (accessed on 1 May 2015).
2. Napmc.com.http://122.170.3.177:9090/napmc/products/cumin.aspx (downloaded on 1 May 2015).
3. Uppal, B. N., Patel, M. K. and Kamat, M. N., *Alternaria* blight of cumin. *Indian J. Agric. Sci.*, 1938, **8**, 49–62.
4. Simmons, E. G., *Alternaria* taxonomy: current status, view point, challenge. In *Alternaria Biology, Plant Diseases and Metabolites* (eds Chelkowski, J. and Visconti, A.), Elsevier Science, Amsterdam, 1992, pp. 1–35.
5. Benali, S., Mohamed, B., Eddine, H. J. and Neema, C., Advances of molecular markers application in plant pathology research. *Eur. J. Sci. Res.*, 2011, **50**, 110–123.
6. Sharma, M., Deep, S., Bhati, D. S., Chowdappa, P., Selvamani, R. and Sharma, P., Morphological, cultural, pathogenic and molecular studies of *Alternaria brassicae* infecting cauliflower and mustard in India. *Afr. J. Microbiol. Res.*, 2013, **7**(26), 3351–3363.
7. Chillali, M., Ighili-Idder, H., Guillaumin, J. J., Mohammed, C., Escarmant, B. L. and Botton, B., Variation in the ITS and IGS regions of ribosomal DNA among the biological species of European Armillaria. *Mycol. Res.*, 1998, **10**(2), 533–540.
8. Jung, D. S., Na, Y. J. and Ryu, K. H., Phylogenic analysis of *Alternaria brassicicola* producing bioactive metabolites. *J. Microbiol.*, 2002, **40**, 289–294.
9. Vishunavat, K. and Kotle, S. J., *Essentials of Phytopathological Techniques*, Kalyani Publishers, Ludhiana, New Delhi, 2009, 2nd edn, p. 147.
10. Alexopoulos, C. J., Mims, C. W. and Blackwell, M., *Introductory Mycology*, John Wiley, New York, 1996, 4th edn, p. 214.
11. Tuite, J. C., *Plant Pathological Methods: Fungi and Bacteria*, Burgess Publishing Company, Minheapolis, 1969, p. 501.
12. White, T. J., Bruns, T., Lee, S. and Taylor, J., Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (eds Innis, M. A. *et al.*), Academic Press, San Diego, 1990, pp. 315–322.
13. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, 2011, **28**, 2731–2739.
14. Perrier, X. and Jacquemoud-Collet, J. P., DARwin software, 2006; http://darwin.cirad.fr/darwin
15. Rotem, J., *The Genus Alternaria. Biology, Epidemiology, and Pathogenicity*, St Paul, APS Press, 1994.
16. Gemawat, P. D. and Prasad, N., Epidemiological studies on *Alternaria blight* of *Cuminum cymanium* L. *Indian J. Mycol. Pl. Path.*, 1972, **2**, 65–74.
17. Khare, M. N., Tiwari, S. P. and Sharma, Y. K., Disease problems in the cultivation of I. Cumin (*Cuminum cyminum* L.) II. Caraway (*Carum carvi* L.) and their management leading to the production of high quality pathogen free seed. *Int. J. Seed Spices*, 2014, **4**(1), 1–8.
18. Sharma, S. and Pandey, R. N., Survival, epidemiology and management of Alternaria blight of cumin in Gujarat. *BIOINFOLET*, 2013, **10**(2B), 639–642.
19. Lakra, B. S., The role of certain environmental variables in the epidemiology of *Alternaria* blight of cumin. *Plant Dis. Res.* (*Ludhiana*), 2005, **20**, 198–200.
20. Pipliya, B. H. and Jadeja, K. B., Cultural variability and mancozeb sensitivity of different isolates of *Alternaria burnsii*. *Indian J. Mycol. Pl. Path.*, 2008, **38**(1), 121–122.
21. Shekhawat, N., Trivedi, A., Sharma, S. K. and Kumar, A., Cultural, morphological and pathogenic variability in *Alternaria burnsii* causing blight of cumin. *J. Mycol. Pl. Path*., 2013, **43**(1), 80–83.
22. Sharma, S. and Pandey, R. N., Variability among isolates of *Alternaria burnsii* from cumin (*Cuminum cyminum* L.). *BIOINFOLET*, 2012, **9**(4A), 610–616.
23. Agrios, G. N., *Plant Pathology*, Department of Plant Pathology, University of Florida, Academic Press, USA, 1997, 4th edn, p. 65.
24. Rosewich, U. L. and Kistler, H. C., Role of horizontal gene transfer in the evolution of fungi. *Annu. Rev. Phytopathol*., 2000, **38**, 325–363.
25. Masunaka, A., Ohtani, K., Peever, T. L., Timmer, L. W., Tsuge, T., Yamamoto, M., Yamamoto, H. and Akimitsu, K., An isolate of *Alternaria alternata* that is pathogenic to both tangerines and rough lemon and produces two host-selective toxins, ACT and ACR-toxins. *Phytopathology*, 2005, **95**(3), 241–247.
26. Johnson, L. J., Johnson, R. D., Akamatsu, H., Salamiah, A., Otani, H., Kohmoto, K. and Kodama, M., Spontaneous loss of a conditionally dispensable chromosome from the *Alternaria alternata* apple pathotype leads to loss of toxin production and pathogenicity. *Curr. Genet.,* 2001, **40**(1), 65–72.

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