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RESEARCH ARTICLE

Molecular and Cultural Characterization of *Alternaria brassicae* Infecting Cauliflower in Uttar Pradesh, India

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Abstract The Alternaria blight or leaf blight (*Alternaria brassicae*) causes severe damage to cauliflower at curd formation and seed setting stage. Roving survey revealed that the disease incidence on cauliflower in different farmer's fields ranged from 10 to 40 % with an average incidence of 20 % in Uttar Pradesh. Twenty-three (23) isolates of *A. brassicae* were collected from different cultivars in Uttar Pradesh and characterized for cultural, morphological, pathogenic and molecular variations. Based on the pathogenicity, isolates of *A. brassicae* were rated as virulent or less virulent based on the percentage disease incidence on cv. Hajipur local. Most of the isolates showed smooth mycelial growth with circular, irregular margin and without concentric zonation. The colony colour is white, dark brown to light brown and pinkish in white. Significant morphological variations in conidial length, conidial width, and number of horizontal septa were observed in all the isolates. The maximum length of conidia ranged from 150 to 122 μm with 8 to 9 transverse and 2 vertical septation. Further the genetic diversity of isolates based on RAPD-PCR using sixteen random primers were produced on clarity, repeatability and the number of polymorphic bands in all the isolates. Cluster analysis of DNA fragments was performed using NTSYSpc V2.2 based on UPGMA method and Jacard coefficient. Based on the analysis the isolates represented four major groups with 75 % similarity.

Keywords Characterization · *Alternaria brassicae* · Cauliflower

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Introduction

The Alternaria blight or leaf blight disease of cauliflower caused by *Alternaria brassicae* (Berk), Sacc., and *A. brassicicola* (Schw.) Wilt., is one of the most destructive fungal disease causing significant qualitative and quantitative yield loss in cauliflower and cabbage at curd formation and seed setting stage. The disease has a global presence but more prevalent in subtropical and temperate countries. It is very difficult to manage the disease, due to no proven source of resistance reported till date in any of the hosts [1]. The yield loss due to this pathogen is 5–30 % in the entire cauliflower and cabbage growing areas of India [2]. The disease incidence and severity in Uttar Pradesh was 10–40 % and 26 %, respectively [2]. One of the significant aspects of biology of an organism is the morphological and physiological characters of the individuals within a species, which are not fixed. The variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes. Alternaria blight severity and

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incidence on cauliflower and cabbage differs between seasons, regions and individual crops in India [3] due to the existence of variability among geographically similar isolates of *A. brassicae*. The disease is seed borne. Frequent movement of germplasms and hybrid seeds lead to increase in disease pressure. *Alternaria brassicae,* the pathogen of cauliflower is very difficult to differentiate from other *Alternaria* spp., due to their similarity in colony characters and identification. Therefore, the present study was conducted to study the variability among *A. brassica* isolates using molecular markers to assess the genetic relationships among different isolates.

Material and Methods

Survey and Collection of Different Isolates of *A. brassicae*

The roving survey was conducted during 2010–2011 in different farmers' fields at different geographical locations of Uttar Pradesh, India to estimate the incidence of leaf blight disease of cauliflower. The disease incidence of leaf blight (% of plants with leaf blight symptoms) was estimated for each field by visual examination of 1,000 plants following a W pattern (by crossing the rows) [3] as a sampling procedure. Leaf samples from cauliflower showing leaf spot symptoms were collected.

Isolation and Maintenance of *A. brassicae* Isolates

Cauliflower leaves showing leaf blight symptoms were sampled randomly from different cultivars at different farmers' fields in different geographical locations of Uttar Pradesh, India. The isolates of *A. brassicae* were designated based on their place of collection (Table 1). The selected infected spots were washed 3–4 times in sterilized distilled water and then surface sterilized by dipping in 4 % sodium hypochlorite (NaOCl) solution for 1 min, followed by washing with sterilized water for 4–5 times. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm petri dishes containing potato dextrose agar (PDA) and incubated at 25 ± 2 °C for seven days. Thereafter, the pure culture *A. brassicae* was isolated by selecting growing mycelia tip on PDA and aseptically transferred into another petri plate containing PDA medium, where it was grown for 15 days at 23 ± 2 °C in the BOD incubator. On the basis of their conidiophore and conidial morphology the pathogen was identified as *A. brassicae* (Berk.) Sacc. [4] and purified by single spore isolation method. The isolated fungal pathogen cultures were maintained on PDA slants at 4 °C.

Cultural Variability of Different Isolates of *A. brassicae*

The cultural characters were recorded on day 9 of inoculation of all isolates of *A. brassicae*. Characters like mycelial growth, zonation, colony colour, pigmentation on medium and texture were recorded by direct observation of culture-grown petri plates and sporulation was recorded on four tested media by slides of 9-day-old cultures under the microscope. All these isolates were tested for their cultural and morphological variations on potato dextrose agar (PDA), corn meal agar (CMA), malt extract agar (MEA) and oat meal agar (OMA) and two synthetic media i.e. Czapek's Dox agar (CZPA) and Richard's agar (RA). Each treatment (Isolate) was replicated thrice. After solidification of the agar, 5 mm culture bits of each isolate were inoculated onto the above-mentioned nutrient media. These inoculated petri plates were kept in BOD at 25 ± 1 °C for growth. The radial growth was recorded at 3 days after inoculation on the above selective nutrient media.

Morphological Variability of Different Isolates of *A. brassicae*

Ocular and stage micrometer was calibrated by the use of micrometry [5]. Morphological variability of 23 isolates of *A. brassicae* was studied by using nine days-old cultures of all the isolates. Twenty conidia from each slide were examined at 40× magnification of light microscope and measured using ocular and stage micrometer. The conidial morphology (spore body length, spore body width, total transverse septation and total longitudinal septation on spores/conidia) of each isolate was recorded. The average was used to calculate the conidial length, width and number of transverse and longitudinal septa.

Pathogenicity Variability

Pathogenicity test was conducted under controlled conditions in pot experiments using highly susceptible varieties of cauliflower (cv. Hajipur local) in order to confirm the identification of the disease and its causal agent. Seedlings were raised in pots filled with sterilized soil. One-month-old plants were used for inoculation. All the fungal isolates were grown in potato dextrose broth (PDB), and nine days-old broth of *A. brassicae* were filtered through sterilized muslin cloth in a clean test tube aseptically. The spore concentration of 2–3 × 10^8 cfu/ml was adjusted by using a Neubauer Haemocytometer and used for spraying the plants. For maintaining the humidity, a humidifier was set up in the chamber and the temperature was maintained for the development of symptoms. The percentage disease incidence was recorded using standard protocols with modifications [2]. The data was taken three times at

Table 1 List of RAPD primers sequences used in the study

Name of primer	Base sequence (5′-3′)
OPH-1	GGTCGGAGAA
OPC-01	TTCGAGCCAG
OPC-07	GTCCCCACGA
OPA-01	CAGGCCCTTC
OPC-10	TGTCTGGGTG
OPB-01	GTTTCGCTCC
OPC-02	GTGATGGCGT
OPC-03	GGGGGTCTTT
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPC-04	CCGCATCTAC
OPC-05	GATGACCGCC
OPC-06	GAACGGACTC
OPH-04	GGAAGTCGCC
OPC-09	CTCACCGTCC
OPC-10	TGTCTGGGTG

periodical intervals to see the disease progress of leaf blight. Symptoms expressed were studied and the pathogen was re-isolated from the infected leaves. The pathogenicity test as above was repeated twice to confirm the results. Similar experiment was conducted under in vitro conditions using detached leaf technique in a plastic tray to confirm the virulence of different isolates. The trays were incubated in BOD at 25 ± 1 °C. Data was taken at five days and seven days after inoculation (DAI). The size of lesion and diameter were measured. Based on the lesion size and diameter the isolates were grouped in four categories as <10 mm = least virulent, 11–15 mm = moderately virulent, 16–20 mm = virulent, and >20 mm = highly virulent.

Molecular Characterization

Fungal Genomic DNA Purification

Genomic DNA of 23 *A. brassicae* isolates infecting cauliflower was purified by following a modified protocol [6]. For genomic DNA isolation all the fungal isolates were grown on PDB at 25 ± 2 °C for 7 days. The fungal mycelium was harvested by filtration through Whatman No. 1 filter paper and washed with sterile distilled water and dried. Two grams of dried mycelium was crushed in liquid nitrogen with the help of mortar and pestle into fine powder. The powdered mycelium (200 mg) was transferred into a micro centrifuge tube (1.5 ml) and 1 ml 2 % (w/v) CTAB buffer containing 1.4 M NaCl, 20 mM EDTA, 100 Mm Tris–HCl (pH 8), 1 % PVP and 0.1 % β-mercaptoethanol was added to it. The mixture was incubated at 65 °C in water bath for 30 min with intermittent shaking and the entire content was centrifuged at 10,000 rpm for 10 min at 4 °C. The supernatant was transferred to new micro centrifuge tubes (2 ml) and equal volume of chloroform: isoamylalcohol (24:1) was added which was mixed thoroughly and centrifuged at 13,000 rpm for 15 min at 4 °C. The supernatant was re-transferred to new tubes and 2/3 vol. of chilled isopropanol was added and incubated at −20 °C for 2 h. It was then centrifuged at 13,000 rpm for 15 min at 4 °C. The pellet was retained and supernatant was discarded. The pellets were dissolved and the second step was repeated thrice until the supernatant became clear. The supernatant was transferred in separate micro centrifuge tubes and 1/7 volume of 3 M sodium acetate and double volume of chilled absolute ethanol was added and incubated at −20 °C for 30 min. DNA was pelleted by centrifugation at 13,000 rpm for 12 min at 4 °C. The pellet was retained and supernatant was discarded. The pellet was washed with 70 % ethanol by centrifuging at 8,000 rpm for 5 min. Finally the pellet was dissolved in 50 µl buffer and stored at -20 °C for further use. The DNA was dissolved in 1 X TAE buffer (pH 8.0). The quality of the genomic DNA was checked on 0.8 % agarose gel and stored at −20 °C till further use.

PCR Amplification

The PCR amplification was carried out according to the protocol [7]. A total of 25 of 10-base random primer (0.4 µM) (Operon Technologies, USA) (Table 1) were used for amplification of total DNA of twenty-three isolates of *A. brassicae*. The total volume of PCR reaction is 25 µl containing 50 ng genomic DNA, 1XPCR buffer (2.5 µl), 25 mM $Mgcl_2$ (2 µL), 2 mM dNTPs (2µL), 1.5U *Taq* DNA polymerase (Fermentas, Germany), 10 pmol of each primer (synthesized by Sigma Aldrich) and 2 µL of template was taken in a PCR tube. The DNA amplification was performed in a Biorad Thermo-cycler (S-1000) with initial denaturation at 94 °C for 5 min, followed by 35 cycles comprising denaturation at 94 °C for 45 s, primer annealing at 37 °C for 45 s, and primer extension at 72 °C for 45 s, followed by final extension at 72 °C for 10 min. PCR product were electrophoresed (1 h at 80 volts) in 1.2 % Agarose gel in Tris–acetate-EDTA buffer (TAE) at pH 8.0. Gels were stained with ethidium bromide (0.3 µg/ml) and viewed in Gel documentation system (Alpha-Innotech, USA). A negative control, without DNA template, was included in all reactions (Negative control means use of distilled water as control. Other species of *Alternaria* are already used in the reaction). Some standard procedures were adopted to ensure the reproducibility of the results viz. the use of the same thermocycler, *Taq* DNA polymerase from the same producer and the same amount of all the mix components in all the experiments, the inclusion of only strong bands in the analysis and the inclusion of standard DNA samples.

Table 2 Survey of Alternaria blight disease in cauliflower during 2010–2011

S.N.	Place	District	Isolate code	% Incidence
1.	Shibali campus	Azamgargh	Azm-C-1	22–26
2.	Rasara	Ballia	Bl-C-1	15–22
3.	Jaraval Road	Baharaich	Brh-C-1	11–20
4.	Faizabad Road	Barabanki	Bar-C-1	17–28
5.	Manikapur	Basti	Bas-C-1	18–30
6.	Jaddupur	Bhadohi	Bh–C-1	11–20
7.	Bhatpar Rani	Deoria	Deo-C-1	9–24
8.	Akabarpur	Faizabad	Fai-C-1	12–26
9.	Gonda Katchahari	Gonda	Gon-C-1	15–32
10.	Chauri Chaura	Gorakhpur	Gpr-C-1	20–35
11.	Mohamadabad	Ghazipur	Gha-C-1	14–33
12.	Badalapur	Jounpur	Jau-C-1	15–22
13.	Pipra Chouraha	Khalilabad	Kha-C-1	15–35
14.	Sapaha Village	Kushinagar	Kus-C-1	12–30
15.	Bakshi ka talab	Lucknow	Luk-C-1	06–27
16.	Haldharpur	Mau	Mau-C-1	10–32
17.	Chunar	Mirzapur	Mir-C-1	15–30
18.	Laxmiganj	Maharajganj	Mah-C-1	10–36
19.	Raibareili	Raibareili	Rai-C-1	05–23
20.	Dudhi	Sonbhadra	Son-C-1	17–28
21.	Sidhartha Nagar	S.Nagar	Sid-C-1	13–35
22.	Amethi	Sultanpur	Sul-C-1	5–18
23.	IIVR	Varanasi	Vns-C-1	8–27

Data Analysis

PCR amplification products of the 23 *A. brassicae* isolates were scored as presence (1) or absence (0) of bands. The analyses were performed using NTSYS-pc software, version 2.0 [8]. The data matrix was used to calculate Jaccard's similarity coefficient [9] which does not consider the joint absence of a marker as an indication of similarity. A dendrogram was constructed using the unweighted pair-group method analysis (UPGMA). The frequencies of the RAPD fragments were estimated for each of the 23 *A. brassicae* isolates infecting cauliflower.

Results and Discussion

Survey and Incidence of *A. brassicae* in Different Cauliflower Fields

The periodical roving survey was conducted in major cauliflower growing districts of Uttar Pradesh during cropping season (Fig. 1). The disease incidence varied from field to field in different districts. The most predominant disease symptoms observed on leaves were restricted to lower portion of leaves, which are dark brown to black circular spots covering the entire leaves. Later these spots become large, covered with black spores in concentric zonation and gave blighted appearance in cauliflower (Fig. 2).The average incidence of disease ranged from 5 to 36 % in different fields (Table 2). The maximum disease incidence was recorded in Maharajganj as 36 % and minimum in Sultanpur as 18 % (Table 2). The disease incidence on cauliflower in different farmer's fields ranged from 10 to 40 % with the average incidence of 20 % recorded in Uttar Pradesh [2].

Radial Growth of *Alternaria brassicae* Isolates

The radial growth of all 23 different isolates of *A. brassicae* was significantly different on four different semi-synthetic media (PDA, CMA, MEA and OMA) and two synthetic media (CZPA and RA). Radial growth of *A. brassicae* isolates in semi-synthetic media varied from 38.00 to 61.67 mm on PDA, 40.84 to 65.50 mm on CMA, 29.33 to 57.00 mm on MEA, 42.33 to 66.33 mm on OMA (Figs. 3, 4). In two synthetic media it varied from 21.67 to 40.93 mm, on CZPA and 36.67 to 75.93 mm on RA. The Mah-Ab isolate recorded maximum radial growth on all four semi synthetic media and two synthetic media. The minimum radial growth was exhibited by Azm-C-1 isolate (Fig. 4). In all the isolates the maximum growth was recorded on Richard's Agar medium (Table 3). Similarly

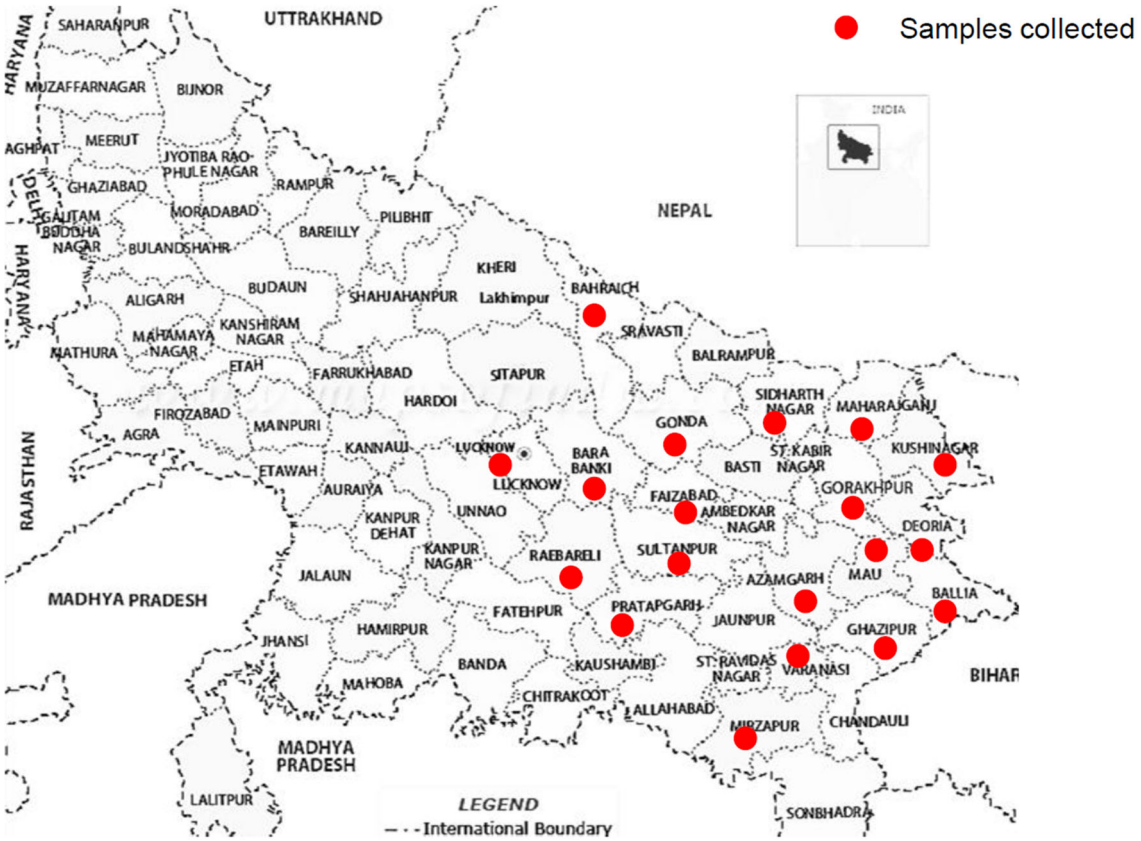


Fig. 1 *Alternaria brassicae* isolates collected from different regions of UP

Fig. 2 Leaf blight symptoms on **A** Upper surface of the leaf, **B** Lower surface of the leaf and **C** Inflorescence

the maximum mycelial growth and sporulation of *A. brassicae* was reported in Richard's medium, both in the solid and liquid state [10].

Conidial Morphology

The length of 20 conidia from each *A. brassicae* isolate was measured in 4 different microscopic fields. The maximum length of conidia ranged from 150 µm (Mah-C1) to 122 µm (Bar-C1). Further, transverse and vertical septation of conidia in each isolate was measured and a maximum of 9 transverse septation in conidia was recorded in Gpr-C1 and Mah-C1 isolates followed by eight transverse septation in Kha-C1 and Mau-C1 isolates. The number of vertical septation in all isolates ranged from 0 to 2. In the present study high level of variability was observed with respect to conidia length, width and septa in all of the *A. brassicae* isolates, which clearly indicates the existence of variability among the 23 isolates (Table 4). Similar results were observed in respect of conidial length, width, and number of septa in *A. brassicae* infecting cauliflower and mustard in India [11]. The conidial variations in *A. brassicae* and *A.*

Table 3 Effect of different media on radial growth of *A. brassicae* isolates

S.N.	Isolates	Radial growth (mm) 10 days after inoculation					
		PDA	CMA	MEA	OMA	CZPA	RA
1.	Azm – C-A	38.00	40.84	29.33	42.33	21.67	36.67
2.	Bl – C-A	40.50	42.70	33.16	42.67	31.33	44.33
3.	Brh – C-A	50.67	52.00	45.16	53.67	31.33	53.93
4.	Bar – C-A	41.66	44.40	37.50	44.33	20.00	41.43
5.	Bas – C-A	51.83	57.00	56.00	57.28	34.00	63.66
6.	Bh – C-A	50.50	56.00	42.20	56.11	24.67	57.27
7.	Deo – C-A	45.40	50.83	40.84	50.84	22.00	56.40
8.	Fai – C-A	45.00	41.00	33.00	45.00	28.33	52.00
9.	Gon – C-A	50.30	45.00	36.17	45.00	21.33	36.67
10.	Gpr – C-A	54.83	63.67	49.67	60.50	37.33	68.30
11.	Gha – C-A	40.00	42.77	30.67	42.66	28.00	43.00
12.	Jau – C-A	51.33	56.33	43.17	56.11	33.00	56.67
13.	Kha – C-A	53.50	57.66	48.33	58.90	36.70	66.40
14.	Kus – C-A	39.33	42.33	36.33	44.34	36.70	55.66
15.	Luk – C-A	51.00	55.33	49.67	56.50	40.90	53.67
16.	Mau – C-A	52.27	57.33	46.33	57.30	35.33	56.00
17.	Mir – C-A	49.00	43.67	48.33	54.67	28.00	49.66
18.	Mah – C-A	61.67	65.50	57.00	66.33	40.93	75.93
19.	Rai – C-A	48.27	50.84	44.70	48.48	27.33	48.00
20.	Son – C-A	49.17	52.66	43.54	53.60	20.67	53.66
21.	Sid – C-A	53.17	57.40	50.50	58.48	35.90	64.33
22.	Sul – C-A	51.70	50.00	47.50	52.93	25.00	50.73
23.	Vns – C-A	50.83	53.33	44.67	55.00	23.00	50.33
CD at 5 %		2.81	4.97	3.39	4.187	3.23	4.31
SE (d)		1.38	2.45	1.67	2.06	1.59	2.12
SE (m)		0.98	1.73	1.18	1.46	1.12	1.50
CV		3.54	6.01	5.19	4.98	7.30	5.11

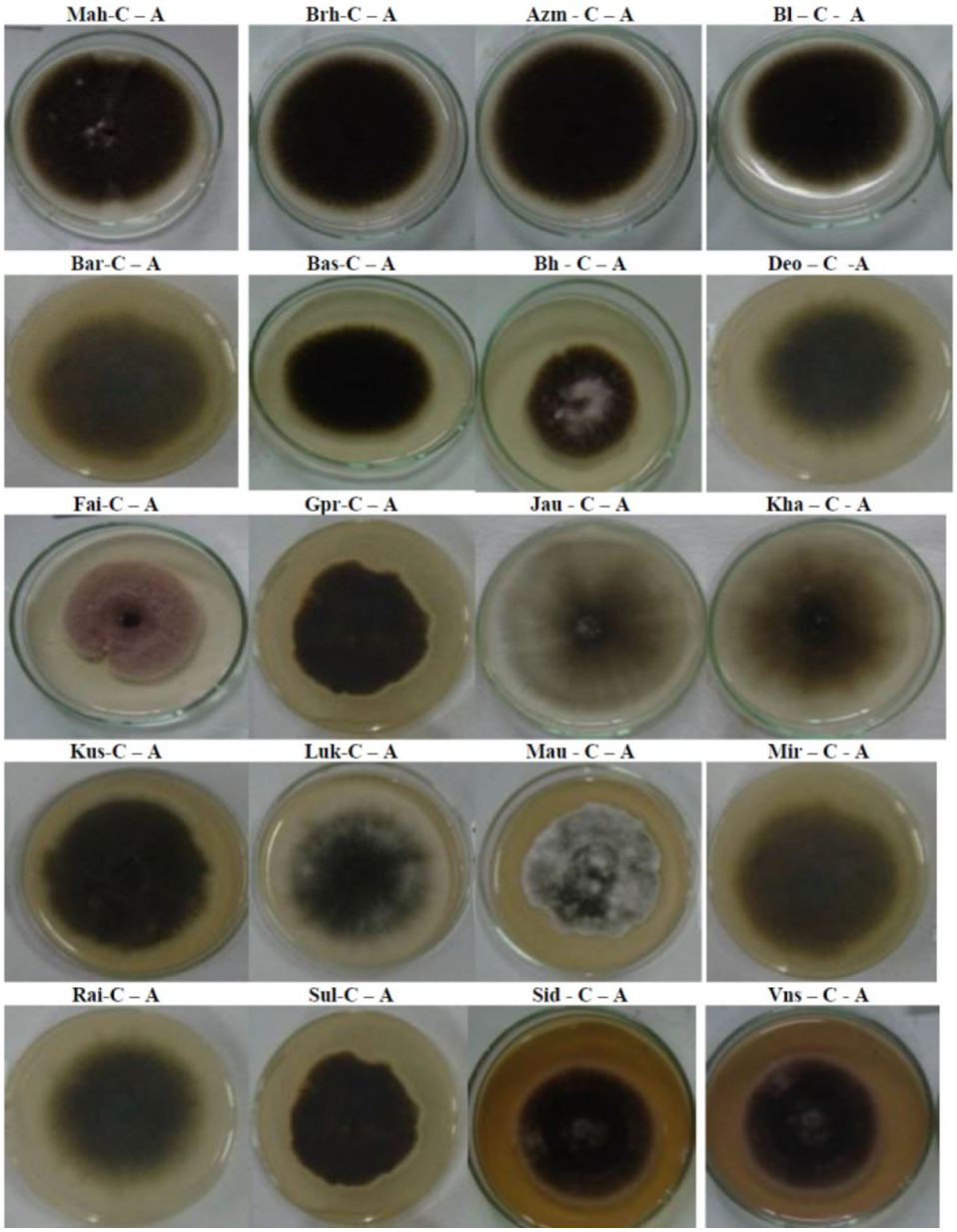
brassicicola are one of the most important criteria to differentiate these two species, but not for distinguishing *A. alternata* from *A. brassicae* [5, 12].

Pathogen Aggressiveness of *A. brassicae* Isolates

The 23 isolates of *A. brassicae* were tested through detached leaf cut method on susceptible variety of cauliflower cv. Hajipur local. On the basis of lesion size, the isolates Mah-C1, Gpr-C1, Kha-C1, Sid-C1 and Bas-C1 were categorized as highly virulent, while, Rai-C1, Azm-C1, Brh-C1, Sul-C1 and Luk-C1 were the virulent ones and Son-C1, Gon-C1, Vns-C1, Bar-C1, Mau-C1 and Deo-C1 were moderately virulent isolates. Others were reported as least virulent or avirulent respectively (Fig. 5) (Table 5). Similar techniques were also followed by other workers [13] to study the pathogenic variability of *A. brassicae* and *A. brassicicola* isolates from three cauliflower varieties of four age groups 15, 30, 45, and 60 days after sowing (DAS) leaves and it was reported that the 2 *A. brassicae* isolates were highly aggressive on 60 DAS Pusa Sharad leaves.

Molecular Variability of *A. brassicae* Isolates

Analysis by twenty-five RAPD primers revealed a high level of genetic variability among twenty-three isolates of *A. brassicae* on different cultivars of cauliflower. Amplification of total DNA of all the *A. brassicae* isolates produced 984 scorable and reproducible RAPD markers. The size of amplified products from all the primers varied between 150 and 3,000 bp. The minimum size of 150 bp amplification products was generated from the primer OPA-03 while the maximum size of 3,000 bp was generated with primer OPC-03, OPC-05, OPC-06 and OPC-10 (Fig. 6). Out of twenty-five RAPD primers, sixteen primers generated on an average 145 amplification products, of which 135 (95.07 %) polymorphic bands were produced.



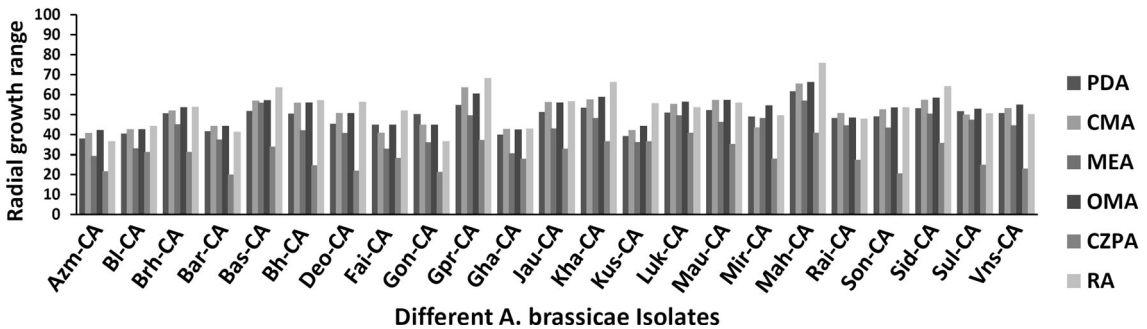


Fig. 3 Colony morphology of *Alternaria brassicae* isolates on PDA

Fig. 4 Variability in radial growth of *A. brassicae* isolates on selected nutrient media (Table 5)

Table 4 Conidial morphology of *Alternaria brassicae* isolates of cauliflower

Isolates	Conidial size			
	Length (μm)	Width (μm)	No. of septa	
			Transverse	Vertical
Azm – C-A	147	24	7	1
Bl – C-A	125	28	4	0
Brh – C-A	125	26	4	0
Bar – C-A	122	23	4	1
Bas – C-A	143	27	7	1
Bh – C-A	125	26	4	0
Deo – C-A	125	23	4	1
Fai – C-A	125	26	4	0
Gon – C-A	123	28	4	0
Gpr – C-A	148	29	9	2
Gha – C-A	139	25	7	1
Jau – C-A	135	23	7	2
Kha – C-A	147	29	8	2
Kus – C-A	134	26	7	1
Luk – C-A	137	24	7	1
Mau – C-A	144	28	8	2
Mir – C-A	137	24	7	1
Mah – C-A	150	30	9	2
Rai – C-A	136	27	6	1
Son – C-A	128	27	4	0
Sid – C-A	143	28	7	1
Sul – C-A	127	28	4	0
Vns – C-A	140	27	7	2
SD	0.89	3.17	2.2	1.05
SE	0.33	1.19	0.83	0.40

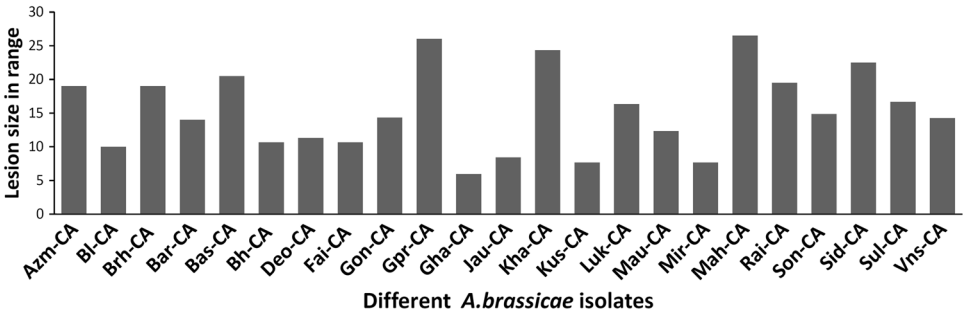


Fig. 5 Variability of *A. brassicae* isolates on Cauliflower (Table 3)

The dendrogram prepared by using the similarity coefficients (Fig. 7) clustered the twenty tree representative isolates into four major groups that is, Group I only 27 % similarity coefficient with other groups (73 % dissimilarity). Group I was composed of three isolates namely, Am-C1, So-C1 and Va-C1, whereas group II was composed of five isolates namely, Az-C1, Gaj-C1, Mau-C-1, Bl-C1 and Gpr-C1 with only 32 % similarity (68 % dissimilarity). Group III composed of four isolates (Fat-C1, Me-C1, Siw-C1 and Ma-C1) having 40 % similarity (60 % dissimilarity) with other groups, while Group IV composed of seven isolates (Bh-C1, Nd-C1, Gag-C1, Ro- C1, Ag-C1, Ut-C1 and Ph-C1) having 46 % similarity (54 % dissimilarity) and Group V composed of only one isolate (Mir-C1). Among the twenty-five RAPD primers used, banding pattern of OPH-01 primer was unique to isolate Ma-C1

Table 5 Cultural, morphological and pathogenic variability of different isolates of *A. brassicae*

Isolates	Pathogen aggressiveness	Mean lesion size (mm)	Pigmentation	Sporulation on different media			Mycelial growth/Colony character		
				PDA	CZPA	RA	Circular/irregular	Smooth/rough	Zonation
Azm-C-1	Virulent	18.95	White	++	+	+++	Adherent, circular	Smooth	Concentric zonation
Bl-C-1	Avirulent	9.5	Dark brown	++	+	++	Fluffy, circular	Smooth	Without zonation
Brh-C-1	Virulent	18.665	Brown	+++	+	+++	Adherent, circular	Smooth	Without zonation
Bar-C-1	Moderate virulent	13.5	Light brown	++	+	+++	Adherent, circular	Smooth	Concentric zonation
Bas-C-1	Highly virulent	20.25	Pinkish white	+++	+	+++	Adherent, circular	Smooth	Concentric zonation
Bh–C-1	Avirulent	10.16	Pinkish white	+++	+	+++	Adherent, circular	Smooth	Without zonation
Deo-C-1	Moderate virulent	11.33	Dark brown	++	+	+++	Fluffy, circular	Smooth	Without zonation
Fai-C-1	Avirulent	10.33	Brown	++	+	++	Adherent, circular	Smooth	Without zonation
Gon-C-1	Moderate virulent	14.165	Pinkish white	++	+	++	Adherent, circular	Smooth	Without zonation
Gpr-C-1	Highly virulent	25.665	Brown	+++	++	+++	Adherent, circular	Smooth	Concentric zonation
Gha-C-1	Avirulent	5.48	Brown	++	+	++	Adherent, circular	Smooth	Without zonation
Jau-C-1	Avirulent	8.38	Dark brown	++	+	++	Fluffy, circular	Smooth	Without zonation
Kha-C-1	Highly virulent	24.165	Brown	+++	++	+++	Adherent, circular	Smooth	Concentric zonation
Kus-C-1	Avirulent	7.16	Brown	++	+	+++	Adherent, circular	Smooth	Without zonation
Luk-C-1	Virulent	15.995	Brown	+	+	+++	Adherent, circular	Smooth	Without zonation
Mau-C-1	Moderate virulent	12.165	Brown	+++	++	+++	Adherent, circular	Smooth	Without zonation
Mir-C-1	Avirulent	8.41	Brown	++	+	++	Adherent, circular	Rough	Without zonation
Mah-C-1	Highly virulent	26.25	Brown	+++	+++	+++	Wavy	Rough	Without zonation
Rai-C-1	Virulent	19.385	Brown	++	+	++	Adherent, circular	Rough	Without zonation
Son-C-1	Moderate virulent	14.43	Brown	++	+	++	Adherent, circular	Rough	Concentric zonation
Sid-C-1	Highly virulent	22.25	Brown	+++	++	+++	Adherent, circular	Rough	Concentric zonation
Sul-C-1	Virulent	16.16	Brown	+	+	+	Wavy	Rough	Without zonation
Vns-C-1	Moderate virulent	13.875	Light brown	++	+	+++	Adherent, circular	Smooth	Without zonation
	SE (d)	0.48							
	SE (m)	0.28							

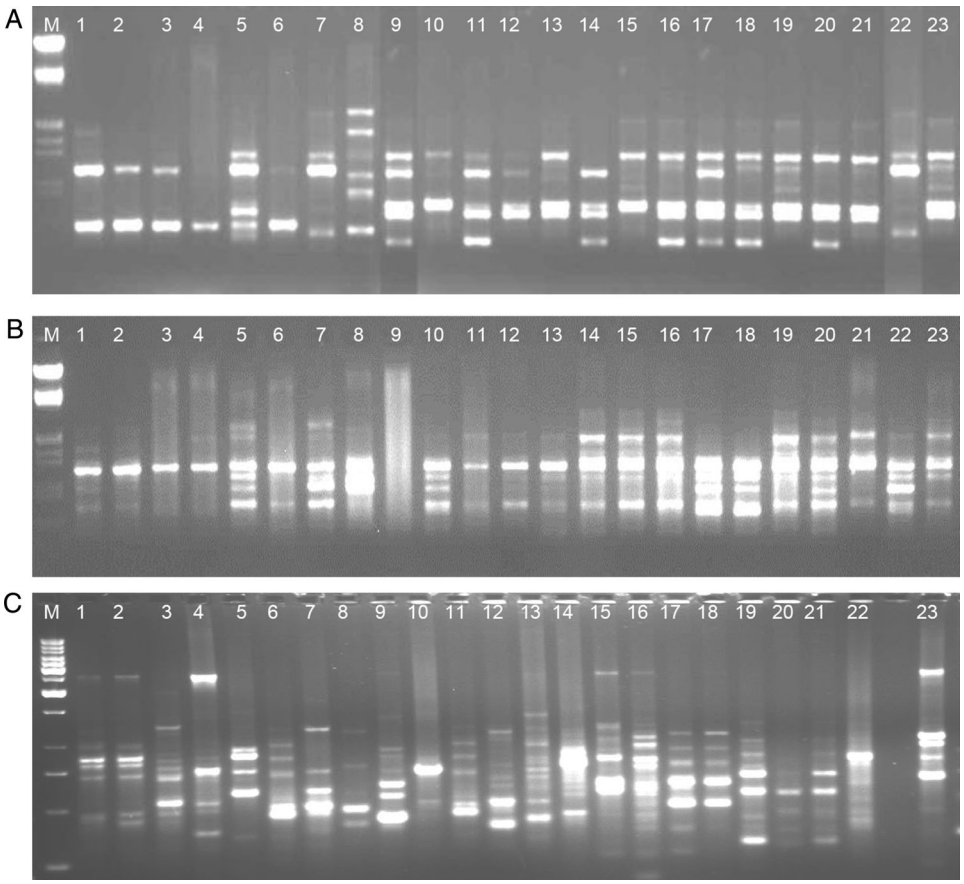


Fig. 6 RAPD profile of *A. brassicae* isolates using random primers **A** OPH-1, **B** OPC-01 **C** OPC-05, *Lane* 1–23 *Alternaria* sp. isolates. *M* Molecular weight marker

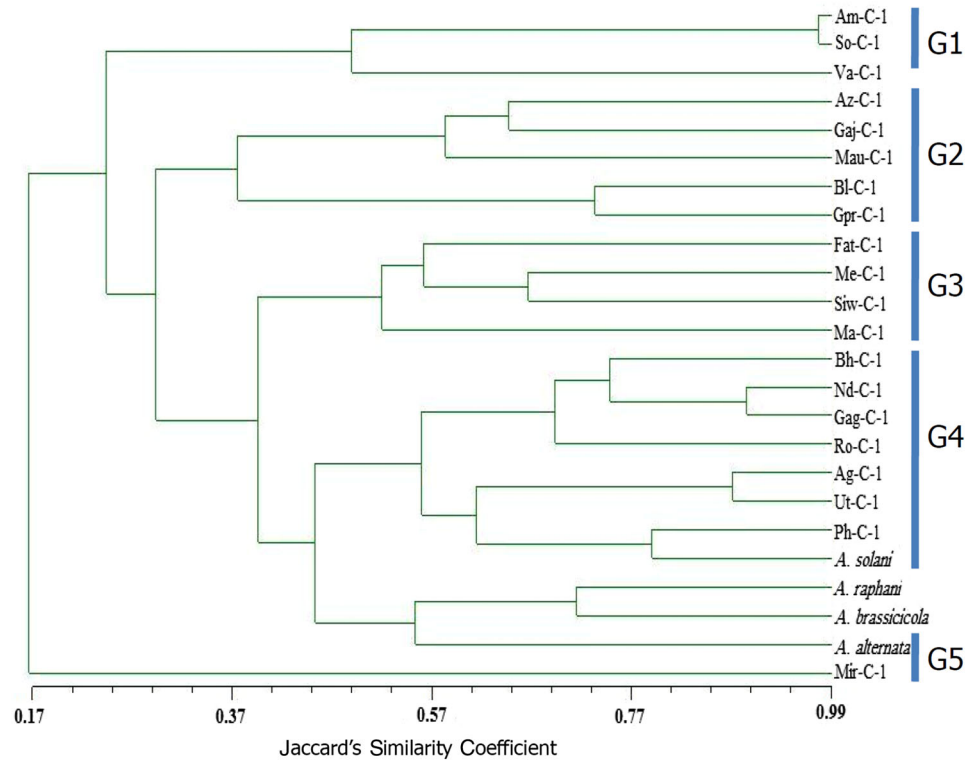


Fig. 7 Genetic divergence among the isolates of *A. brassicae* based on UPGMA cluster analysis

Manikpur (U.P), Bh-C1 Bhagalpur (Bihar), Nd-C1 Azadpur Mandi (New Delhi), Ut-C1 Pantnagar (Uttarakhand) and Ph-C1 Panchkulla (Haryana). The present results indicated high genetic divergence among the twenty-three isolates of *A. brassicae* isolated from cauliflower. The genetic polymorphism within an *Alternaria* species have been successfully studied using RAPD molecular markers by many workers [14–16]. The genetic polymorphism among *A. brassicae* isolates from different geographical regions of the world was studied and low intra-regional variation among Indian and Canadian isolates of *A. brassicae* with 75 % similarity among them was reported [14]. This genetic variability within a species of *Alternaria* might be due to the existence of heterokaryosis, mutation, somatic hybridization, host selection, extensive dispersal or of a cryptic sexual stage. High degree of genetic variability was observed among twenty-three isolates of *A. brassicae* isolated from different cultivars of cauliflower growing in eastern parts of Uttar Pradesh. This could be the probable reason behind extreme and different disease reactions of genotypes at most of locations.

Future Perspective

Alternaria brassicae is one of the most destructive fungal pathogens causing blight on cruciferous crops. The disease is more prevalent in subtropical and temperate countries. This adaption may be due to the existence of wide variability in pathogen. To understand the details of the gene functionality in pathogen under such conditions, in-depth study like characterization of internal transcriber spacer region, toxins and interaction of different varieties of cauliflower to the pathogen needs to be studied. Their survival in the off season on different alternate host is an important point of investigation. The present study is the cornerstone for such host-pathogen interaction studies which can be exploited to contain the disease in an effective manner.

Conclusion

The study highlights the wide range of variability of *A. brassicae* in cauliflower causing blight disease. In order to provide a better picture of the pathogenic as well as genetic divergence among *A. brassicae* populations of India, there is a need to conduct similar holistic investigation among higher number of *A. brassicae* isolates which could be helpful to generate resistant material against Alternaria blight. On the other hand, RAPD is a valuable tool for detecting pathogen variation in the absence of a standard set of differential hosts.

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