PLANT SCIENCE TODAY
Vol 13(sp1): 1-10
https://doi.org/10.14719/pst.10575

eISSN 2348-1900

RESEARCH ARTICLE

Isolation and characterisation of *Alternaria brassicae* (Berk.) Sacc from *Brassica* spp. from diverse agro-climatic zones of India

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Received: 10 July 2025; Accepted: 13 October 2025; Available online: Version 1.0: 05 March 2026

Cite this article: Singh HK, Manish KM, Divya S, Shubham P, Ajay K, Vikas Y, Mishra DS, Prakash Y, Anand S, Aina Y, Simran. Isolation and characterisation of *Alternaria brassicae (Berk.) Sacc* from *Brassica* spp. from diverse agro-climatic zones of India. Plant Science Today. 2026; 13(sp1): 1-10. https://doi.org/10.14719/pst.10575

Abstract

Alternaria brassicae (Berk.) Sacc, a necrotrophic pathogen, is responsible for up to 48 % yield loss in rapeseed-mustard. Therefore, a study was carried out at the laboratories of the Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (UP), during Rabi season 2019–20 and 2020–21. In this study, ten isolates of A. brassicae were collected from infected leaf samples across different Indian states and evaluated for cultural and morphological variability. Significant differences were observed among isolates with respect to conidiophore and conidial traits, septation and growth on various media. Conidiophore length ranged from 33.28 µm (Abr_2) to 66.44 µm (Abr_7), while width varied from 4.83 µm (Abr_1) to 7.72 µm (Abr_3). Septation in conidiophores was highest in Abr_3 (6.66) and lowest in Abr_4 (4.00). Conidial dimensions also showed variability, with maximum length (146.45 µm), width (18.36 µm) and beak length (73.46 µm) recorded in Abr_3, whereas Abr_2 exhibited the lowest measurements. Horizontal septa ranged from 5.66 (Abr_2) to 8.66 (Abr_6, Abr_7) and longitudinal septa ranged from 0.33 (Abr_2) to 2.66 (Abr_3). Mycelial growth assessment on five solid media, viz. PDA, oatmeal agar, host leaf extract agar, czapek dox agar and carrot juice agar, revealed isolate-specific responses. Abr_3 consistently showed the highest radial growth on PDA (90.00 mm), czapek dox (55.64 mm) and carrot juice agar (76.78 mm), while Abr_2 and Abr_{10} exhibited minimal growth. Colony colour varied from light brown to black across media, with appearance ranging from compressed to fluffy and margins from smooth to wavy. This variability among *A. brassicae* isolates is critical for understanding pathogen diversity and for resistance breeding strategies in Brassica crops.

Keywords: *Alternaria brassicae*; isolation; PDA (potato dextrose agar); rapeseed-mustard; variability

Introduction

In India, rapeseed-mustard is an important group of edible oilseed crops. *Alternaria brassicae* (Berk.) Sacc. is an important necrotrophic pathogen causing *Alternaria* blight disease in Indian mustard [*Brassica juncea* (L.) Czern and Coss.] (1, 2). Rapeseed-mustard is the most important oilseed crop, accounting for nearly 31 % of the total oilseed production in the country (3, 4). India has about 88.06 lakh ha area, 124.94 lakh tonnes of production annually, with an average yield of 1419 kg/ha (5).

The constraints in growing oilseed mustard crop are diseases, aphid pests, weeds and abiotic stresses, including frost injury and salt, which are responsible for reduction in growth, yield and oil production (6–10). Among these, diseases are major constraints, of which Alternaria blight caused by *A. brassicae*. and *A. brassicicola* (Schw) Wiltshire is one of the most severe and yield destabilising factors, reducing up to 70 % (10–12). Alternaria blight is a polycyclic disease, with many infections occurring throughout the growing season (13). It is a foliar pathogen and causes a relatively slow destruction of host tissues through the reduction of photosynthetic potential (14). The disease also adversely affects quality by reducing seed size, impairing seed colour and oil content (15). In oilseed Brassicas, the symptoms of disease caused by *A. brassicae* appear primarily in the seedling stage on cotyledons and hypocotyls in the form of small light brown lesions, which at the adult stage affect leaves, leaf petiole, stem, inflorescence, siliquae and seeds (16, 17).

Chemical control is the only option for its management because complete resistance is lacking in Brassica species all over the world against this fungus (18). Therefore, the resistant cultivars are required to stabilise the productivity and to promote sustainability without using hazardous chemical control in oilseed Brassica crops. Variability studies are important to document the changes occurring in populations and individuals, as variability in morphological and physiological traits indicates the existence of different pathotypes (19). The morphological and cultural characteristics within the isolates of *A. brassicae* have been reported by some workers (20). *Alternaria* blight severity on oilseed Brassicas differs season to season, region to region and also individual crop to crop in India (21). This might be due to the existence of variability among geographically similar isolates of *A. brassicae*. The variability is a well-known phenomenon in genus *Alternaria* and may be noticed as changes in spore shape and size, growth and sporulation, pathogenicity, etc. Diversity appears even in single spore isolates. The present study was conducted to find out the variability in *A. brassicae.*

Materials and Methods

Study site

The Experiment was carried out at the laboratories of the Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.), during the Rabi season 2019–20 and 2020–21. The experimental site is geographically situated in the Indo-Gangetic plains of Eastern Uttar Pradesh at a latitude of 26.47° N, a longitude of 82.12° E and an altitude of 113 m above the sea level (Fig. 1).

Collection, isolation and purification

Ten isolates of *A. brassicae* were collected from various agro-climatic regions of India during the Rabi season (Fig. 2). The samples were kept in dry paper envelopes, especially meant for this purpose. Envelops were marked clearly mentioning the location, variety and date of collection (Table 1). The collected samples were dried for 24 hr under shade to remove excess surface moisture in the laboratory and then the samples were kept in a B.O.D. incubator at 6–8 °C for further study. The present sample was cultured on PDA and further purification was done using single spore isolation. The culture was preserved in the refrigerator (4 °C) for further studies.

The pathogen was isolated from infected leaves or pods of *Brassica juncea* and *B. rapa* exhibiting typical symptoms of Alternaria leaf spot by *A. brassicae*. These selected spots were washed 3–4 times in sterilised distilled water and then surface sterilised by dipping in 4 % NaOCl solution for 1 min, followed by washing with sterilised water 3–4 times. Surface-sterilised leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing potato dextrose agar (PDA) and incubated at 23 ± 2°C for seven days. Thereafter, growing mycelia from the margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another Petri plate containing PDA medium, where they were 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* and purified by the single spore isolation method (22). The isolated fungal pathogen cultures were maintained on PDA slants at 4 °C.

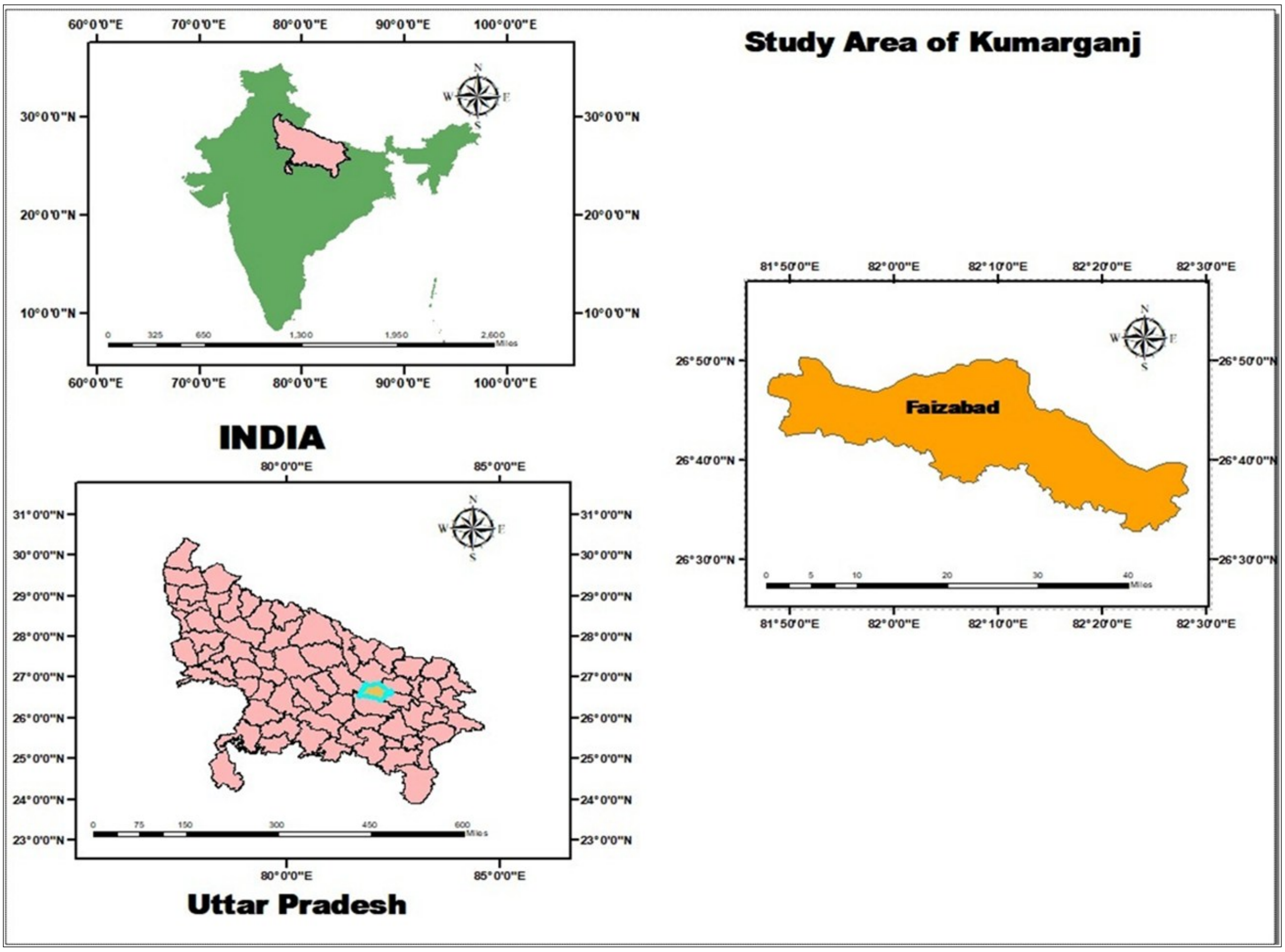

Fig. 1. Experiment site and location.

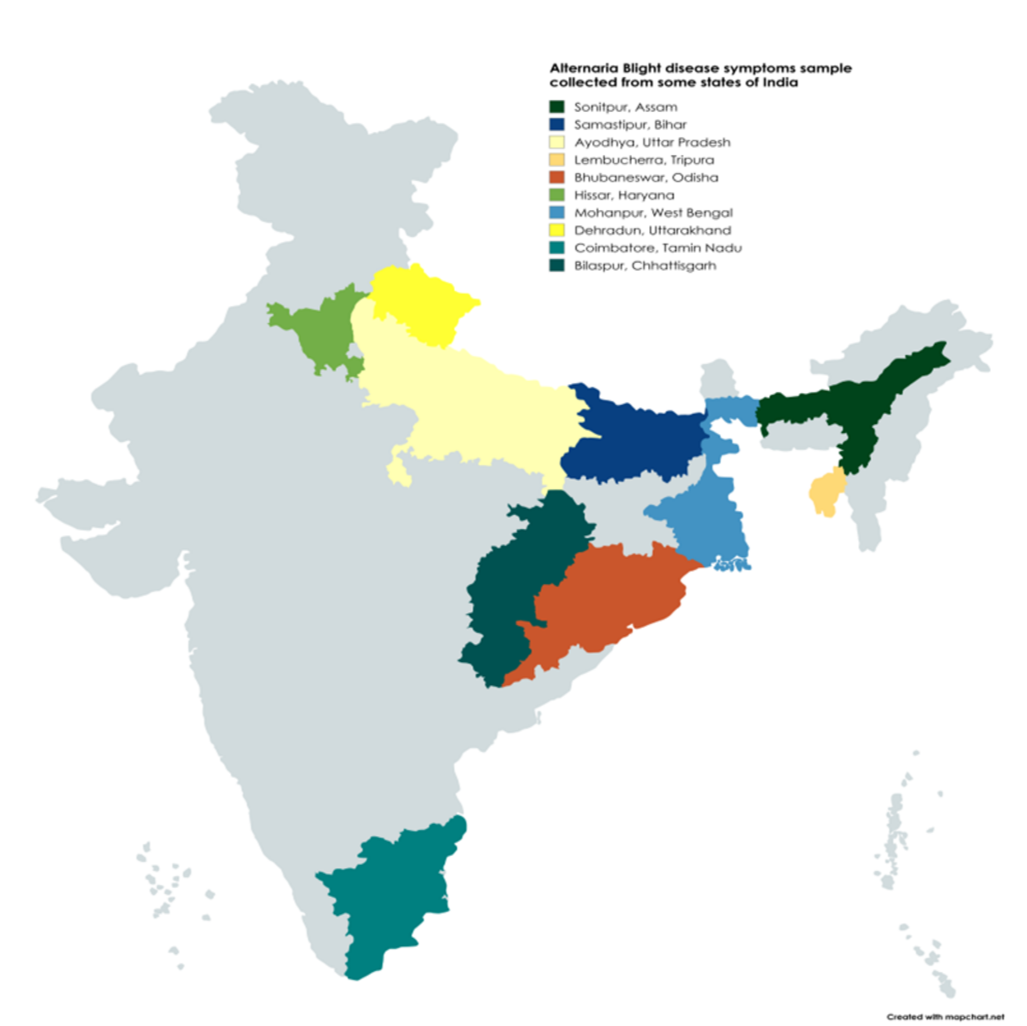

Fig. 2. Collection site of Alternaria blight disease.

Table 1. Diseased samples collected from different places in India

Isolate No.	Place of collection	Host	Plant part
Abr_1	Sonitpur (Assam) 26.67° N, 9285° E	*Brassica juncea*	Leaf
Abr_2	Samastipur (Bihar) 25.85° N, 85.78° E	*Brassica rapa*	Leaf
Abr_3	Ayodhya (Uttar Pradesh) 26.70° N, 82.13° E	*Brassica rapa*	Leaf
Abr_4	Lembucherra (Tripura) 23.90° N, 91.31° E	*Brassica juncea*	Leaf
Abr_5	Bhubaneswar (Odisha) 20.29° N, 85.82° E	*Brassica juncea*	Leaf
Abr_6	Hisar (Haryana) 29.14° N, 75.72° E	*Brassica juncea*	Leaf
Abr_7	Mohanpur (West Bengal) 21.83° N, 87.42° E	*Brassica juncea*	Leaf
Abr_8	Dehradun (Uttarakhand) 30.31° N, 78.03° E	*Brassica juncea*	Leaf
Abr_9	Coimbatore (Tamil Nadu) 11.01° N, 76.95° E	*Brassica juncea*	Leaf
Abr_{10}	Bilaspur (Chhattisgarh) 22.07° N, 82.14° E	*Brassica juncea*	Leaf

Cultural variability

Growth, their appearance, colony colour, margin and zonation of 10 isolates of *A. brassicae* grown on five different culture media viz. Potato dextrose agar, Host leaf extract agar, Oatmeal agar, Czapeks' Dox Agar and Carrot juice agar were observed in three replications from 10-day-old cultures. The observations on radial growth (mm), colony colour, shape, margin, zonation number and appearance of each isolate on different media were recorded 10 DAI at 24 ± 1 °C.

Morphological variability

Morphological variability of 10 isolates studied on the basis of different characteristics, *viz.,* size and septation of conidiophores, conidia and conidial beak, was studied on PDA medium incubated at 25 °C ± 2°C for 15 days. Growth of each isolate was measured as colony diameter in mm. The size of conidia, conidiophores and conidial beak was examined under a microscope using ocular micrometry (23). Observation of conidial septation was done by using a light microscope (40X), taking 20 conidia from each slide. The average was used to calculate the conidiophores and conidial length, width, beak length and number of septa.

Statistical analysis

All the data for each character under study were statistically analysed, following the procedure of a complete randomised design and calculations were done after applying the test of significance for the treatment means. The data obtained on the percentage was transformed into angular values (24).

Results

Morphological variability

The different *A. brassicae* isolates showed significant morphological variability with respect to the pathogen was identified based on morphological characters such as shape, size and septation of conidiophores and conidia on PDA media (Fig. 3). The length of conidiophores ranged from 33.28 to 66.44 µm among different isolates of *A. brassicae*. The data presented in Table 2 and Fig. 4 revealed that the conidiophore length was maximum in Abr_7 (66.44 µm). However, it was found minimum (33.28 µm) in isolate Abr_2.

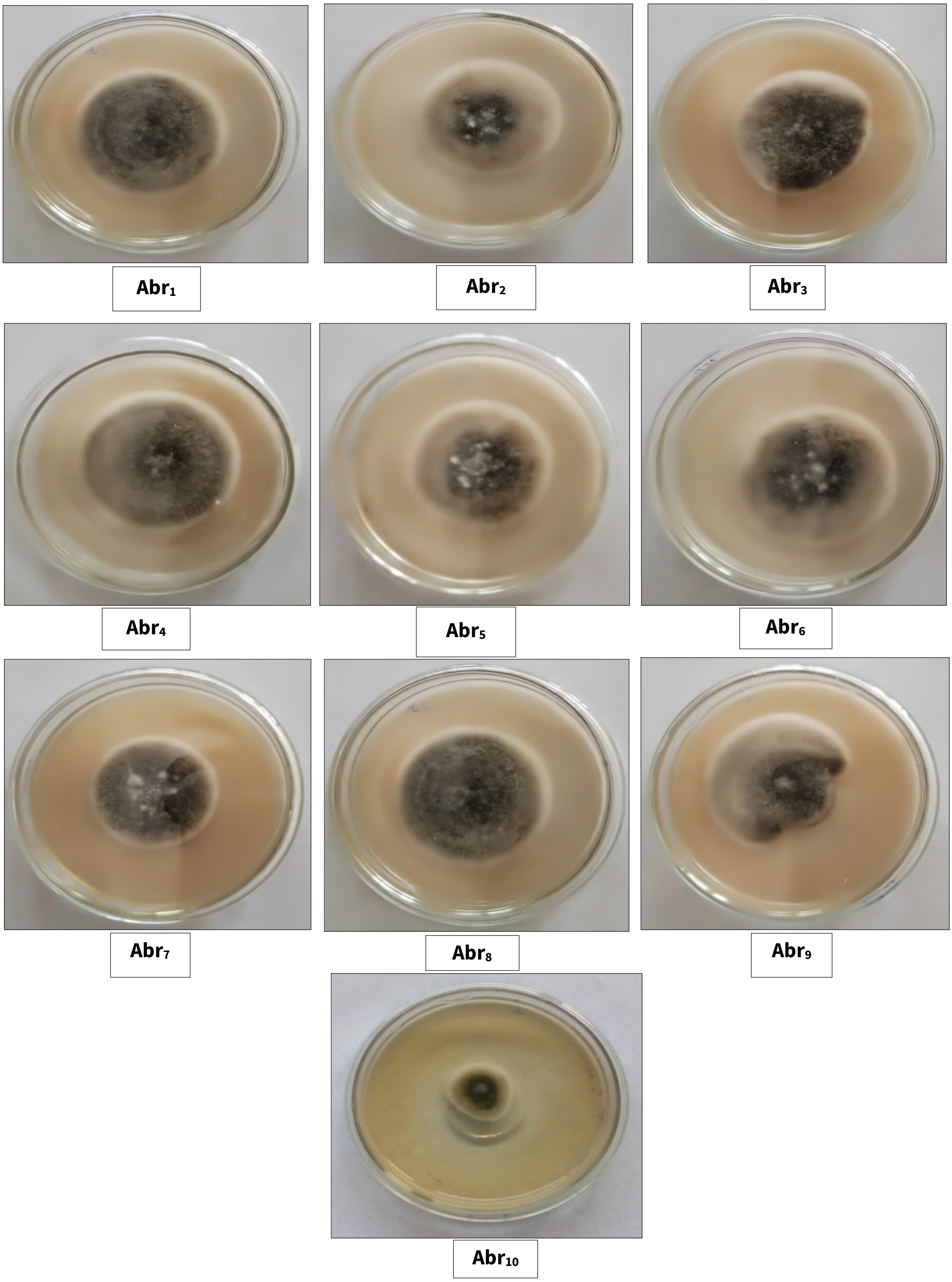

Fig. 3. Different isolates of *A. brassicae* in PDA (Potato dextrose agar) media.

Table 2. Size and septation of conidiophores in different isolates of *A. brassicae*

Isolates	Conidiophores		
	Length (µm)	Width (µm)	Number of septa
Abr_1	38.12	4.83	4.33
Abr_2	33.28	5.68	5.00
Abr_3	64.74	7.72	6.66
Abr_4	41.74	5.12	4.00
Abr_5	40.62	6.34	5.00
Abr_6	53.12	7.28	5.66
Abr_7	66.44	7.42	6.33
Abr_8	46.54	6.58	5.33
Abr_9	37.78	6.40	4.66
Abr_{10}	35.52	5.28	4.00
Average	45.79	6.26	5.09
SEm±	1.48	0.25	0.20
CD at 5 %	4.37	0.74	0.59
C.V. (%)	5.60	6.92	6.89

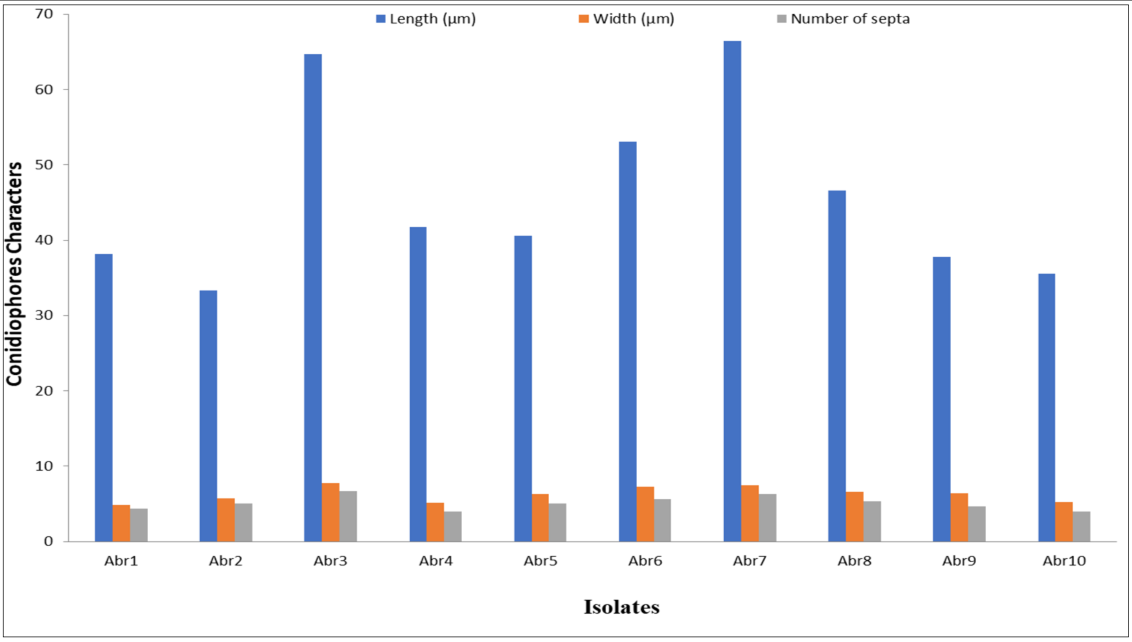

Fig. 4. Size and septation of conidiophores in different isolates of *A. brassicae.*

The width of conidiophores ranged from 4.83 to 7.72 µm. The conidiophores' width was maximum (7.72 µm) on isolate Abr_3, while the minimum (4.83 µm) conidiophore width was recorded in isolate Abr_1. The septation of conidiophores ranged from 4.00 to 6.66. The highest number of septa was found in isolate Abr_3 (6.66), whereas isolate Abr_4 showed the minimum (4.00) number of septa.

The data presented in (Table 3) and (Fig. 5) revealed that the conidial length (µm) varied from 109.48 µm to 146.45 µm maximum in Abr_3 (146.45 µm), while Abr_2 showed the lowest length (109.48 µm). In terms of conidial width ranged from 13.34 µm to 18.36 µm. The conidial width maximum was recorded in isolate Abr_3 (18.36 µm) and Abr_2 (13.34 µm) showed the minimum conidial width. The beak length of conidia fluctuated from 48.46 µm to 73.46 µm. The maximum beak length was found in isolate Abr_3 (73.46 µm), while the minimum was in isolate Abr_{10}(48.46 µm). The transverse septation of conidia varied from 5.66 to 8.66. The highest number of horizontal septa was shown by isolate Abr_7 and Abr_6 (8.66), whereas isolate Abr_2 (5.66) showed the minimum. The longitudinal septa ranged from 0.33 to 2.66. The longitudinal septa were maximum in isolate Abr_3 (2.66). It was observed to be minimum in isolate Abr_2 (0.33).

Table 3. Size and septation of conidia in different isolates of *A. brassicae*

Isolates	Conidial characters				
	Length (µm)	Width (µm)	Beak length (µm)	Transverse septa	Longitudinal septa
Abr_1	117.73	14.34	56.72	7.00	1.33
Abr_2	109.48	13.34	54.32	5.66	0.33
Abr_3	146.45	18.36	73.46	7.60	2.66
Abr_4	113.29	15.76	53.98	8.00	0.66
Abr_5	130.72	16.92	59.48	7.30	2.33
Abr_6	140.68	17.95	68.14	8.66	2.00
Abr_7	143.52	17.80	71.12	8.66	2.00
Abr_8	115.36	14.07	62.76	6.00	1.00
Abr_9	125.79	14.12	51.73	8.30	1.66
Abr_{10}	120.12	14.68	48.46	6.66	1.00
Average	126.31	15.73	60.01	7.38	1.49
SEm ±	3.71	0.62	2.36	0.28	0.06
CD at 5 %	9.78	1.85	6.97	0.85	0.18
C.V. (%)	4.54	6.91	6.82	6.75	7.34

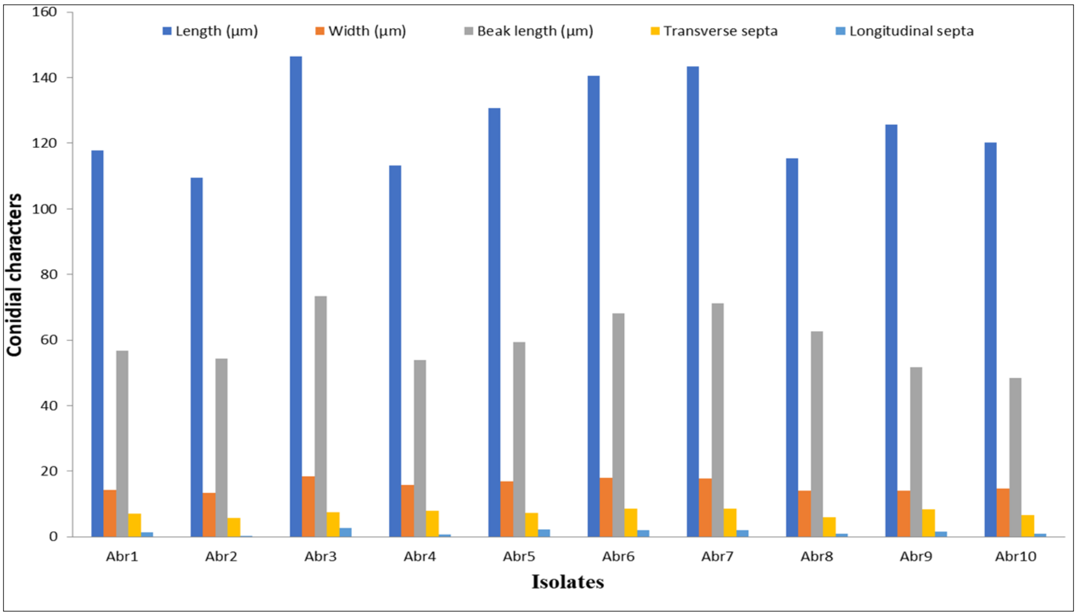

Fig. 5. Size, diameter and septation of conidiophores in different isolates of *A. brassicae*.

Cultural variability

Isolates of *A. brassicae* showed variable cultural characteristics like growth, appearance, colony colour, margin and zonation of ten isolates of *A. brassicae* observed from 10-day-old culture, which was grown on five different culture media, viz., potato dextrose agar, oatmeal agar, host leaf extract agar, czapek-dox-agar and carrot juice agar. Ten isolates of *A. brassicae* were grown on five different media for cultural characteristics. The mycelial growth (mm) on PDA ranged from 72.72 to 90.00 mm in different isolates of *A. brassicae*. The data presented in Table 4 and Fig. 6 revealed that the mycelium growth was maximum in isolate Abr_3 (90.00 mm) and minimum mycelium growth was observed in isolate Abr_2 (72.72) on PDA. The growth of mycelium on oatmeal agar ranged from 69.12 mm to 87.06 mm. The mycelium growth was maximum in isolate Abr_7 (87.06) and it was minimum in isolate Abr_2 (69.12). The mycelial growth ranged from 68.92 to 88.64 mm on Host leaf extract agar. It was maximum in isolate Abr_6 (88.64) and minimum in isolate Abr_2 (68.92). In czapek dox agar, mycelial growth ranged from 36.32 to 55.64 mm. The mycelial growth was maximum in isolate Abr_3 (55.64) and it showed minimum in Abr_{10} (36.32). The mycelial growth on carrot juice agar ranged from 59.18 to 76.78 mm. Maximum growth was observed in isolate Abr_3 (76.78) and minimum in isolate Abr_{10} (59.18). The effect of different media on the growth of ten isolates of *A. brassicae* indicates that maximum growth in all the isolates was noted on PDA, followed by host leaf extract, oatmeal agar and carrot juice agar. Minimum growth over the isolates was found on czapek-dox-agar.

Colony colour, appearance, growth, margin and zonation were noted on 3 different media (potato dextrose agar, oatmeal agar, host leaf extract agar). These characters are one of the important parameters deciding variability among the species. The differences in colour of the colony were observed among all ten isolates of *A. brassicae* showed variations among different media (Table 5). The colour of colonies varies between light brown to black on PDA, Oatmeal agar. Whereas, the Host leaf extract agar showed white, brown, to black colony colours of different isolates. The colony appearance and margin were recorded as compressed to fluffy and smooth to wavy among different media for all isolates. The colony growth of *A. brassicae* was recorded as medium to fast in PDA, oatmeal agar and host leaf extract agar.

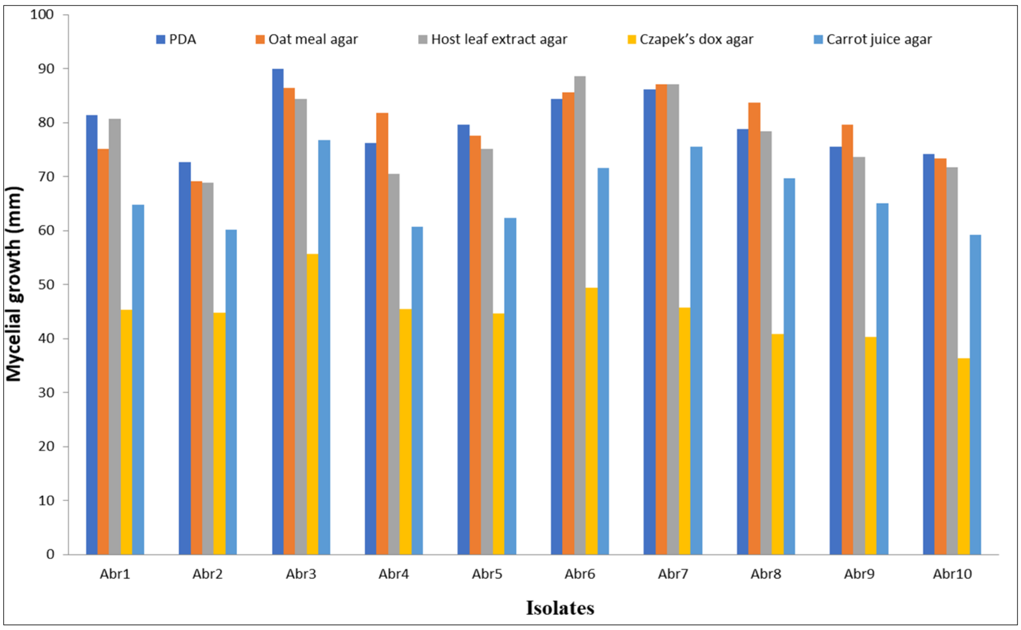

Fig. 6. Effect of different solid media on mycelial growth (mm) of *A. brassicae* isolates.

Table 4. Effect of different solid media on mycelial growth of *A. brassicae* isolates

Isolates	Mycelial growth (mm)				
	PDA	Oat meal agar	Host leaf extract agar	Czapeks' dox agar	Carrot juice agar
Abr$_1$	81.42	75.13	80.68	45.33	64.79
Abr$_2$	72.72	69.12	68.92	44.83	60.14
Abr$_3$	90.00	86.48	84.34	55.64	76.78
Abr$_4$	76.18	81.78	70.54	45.48	60.76
Abr$_5$	79.68	77.56	75.14	44.68	62.34
Abr$_6$	84.32	85.62	88.64	49.38	71.63
Abr$_7$	86.12	87.06	87.12	45.68	75.59
Abr$_8$	78.76	83.68	78.34	40.78	69.66
Abr$_9$	75.52	79.68	73.68	40.28	65.11
Abr$_{10}$	74.16	73.32	71.67	36.32	59.18
Average	79.88	79.94	77.89	44.84	66.59
SEm±	3.16	3.12	3.09	1.77	2.61
CD at 5 %	9.32	9.23	9.14	5.23	7.71
C.V. (%)	6.85	6.77	6.88	6.84	6.79

Table 5. Effect of different solid media on cultural characteristics of *A. brassicae* isolates

Isolates	Cultural characters														
	PDA					Oat meal agar					Host leaf extract				
	Growth	Appearance	Margin	Zonation	Colour	Growth	Appearance	Margin	Zonation	Colour	Growth	Appearance	Margin	Zonation	Colour
Abr$_1$	Fast	Compressed	Wavy	Present	Black	Fast	Compressed	Smooth	Present	Black	Fast	Compressed	Wavy	Absent	Dark brown
Abr$_2$	Medium	Compressed	Smooth	Absent	Brown	Medium	Compressed	Smooth	Present	Dark brown	Fast	Compressed	Smooth	Present	Dark brown
Abr$_3$	Fast	Fluffy	Smooth	Absent	Black	Fast	Compressed	Smooth	Present	Brown	Fast	Fluffy	Rough	Present	Black
Abr$_4$	Medium	Compressed	Wavy	Present	Brown	Medium	Compressed	Smooth	Absent	Brown	Medium	Compressed	Smooth	Present	Brown
Abr$_5$	Medium	Fluffy	Smooth	Present	Brown	Medium	Compressed	Smooth	Present	Brown	Fast	Fluffy	Smooth	Absent	White brown
Abr$_6$	Medium	Compressed	Wavy	Absent	Black	Fast	Fluffy	Smooth	Absent	Black	Fast	Compressed	Smooth	Absent	Black
Abr$_7$	Fast	Fluffy	Smooth	Present	Black	Fast	Compressed	Smooth	Absent	Black	Fast	Smooth	Smooth	Present	Dark brown
Abr$_8$	Fast	Fluffy	Wavy	Present	Black	Medium	Compressed	Smooth	Present	Brown	Fast	Fluffy	Wavy	Present	Dark brown
Abr$_9$	Medium	Compressed	Smooth	Absent	Light brown	Fast	Compressed	Smooth	Absent	Brown	Medium	Compressed	Smooth	Absent	Brown
Abr$_{10}$	Medium	Compressed	Wavy	Present	Light brown	Medium	Compressed	Smooth	Present	Light brown	Medium	Fluffy	Smooth	Present	White brown

Discussion

The observed morphological variability among the *A. brassicae* isolates aligns with previous studies highlighting significant differences in conidial and conidiophore characteristics. Research indicates that substantial variations in spore morphology among *A. brassicae* isolates, with conidial lengths ranging from 29.0 to 185.3 µm and widths from 6.6 to 28.2 µm (25). These findings are consistent with the current study, where conidial lengths varied from 109.48 to 146.45 µm and widths from 13.34 to 18.36 µm. These measurements were found to be within the range of the conidiophores and conidia of *A. brassicae* described by various earlier workers, who observed morphological variability in different geographical isolates within *Alternaria* species (26).

Variations in conidiophore dimensions have also been documented. Earlier studies found that conidiophore lengths ranged from 49.7 to 82.0 mm (25). In the present study, conidiophore lengths ranged from 33.28 to 66.44 µm and widths from 4.83 to 7.72 µm, further corroborating the variability reported in earlier research. The number of septa in conidia and conidiophores also exhibited variability. Research indicates that horizontal septa range from 3.50 to 14.75 and vertical septa from 0.75 to 5.0 (27). In this study, transverse septation in conidia varied from 5.66 to 8.66 and longitudinal septa ranged from 0.33 to 2.66, aligning with previous findings. The morphological variability in different geographical isolates within *A. brassicae* was noticed (28–29).

The present finding emphasises the significant variation in the radial growth of *A. brassicae* isolates when cultured on different artificial media, understanding the critical role of nutrient composition in determining fungal physiological stages. However, among the tested media, Potato Dextrose Agar (PDA) supported the most prolific mycelial development, with growth approaching the maximum quantifiable colony diameter. Similar growth patterns with maximum radial extension (90.00 mm) on PDA were noticed, which was followed by Host Leaf Extract Agar (87.00 mm) and Oatmeal Agar (71.66 mm) (10, 30, 31).

The better performance of PDA is largely attributed to its rich carbohydrate profile derived from potato infusion and dextrose, which provides desired nutrients that promote vigorous hyphal growth and dense mycelial formation and development. Its complex organic matrix mimics the nutrient conditions found in decaying plant matter, favouring rapid vegetative growth of necrotrophic pathogens such as *A. brassicae* (32). Host Leaf Extract Agar, which supported considerable mycelial growth, contains plant-derived metabolites that provide conditions of the natural host environment and support that *A. brassicae* is well adapted to utilise host-specific substrates. Oatmeal Agar, less than PDA or leaf extract media, supported moderate fungal growth (33).

Collectively, these findings illustrated that the type of culture medium significantly influences the radial growth and the morphological features of *A. brassicae*. For this, the detailed advanced studies required for mycelial growth and morphological characterisation, although PDA is still recommended as the standard medium. Host leaf extract agar may be better for simulating *in vivo* conditions, whereas oatmeal agar is considered for pigmentation and sporulation studies. Therefore, a careful selection of media is required based on research objectives for meaningful outcomes in fungal biology research (34).

The growth and morphology of *A. brassicae* are considerably governed by the composition of the culture media. In the present study, minimal growth was recorded on Czapek-Dox Agar (CDA), a finding consistent with previous research indicating that CDA is suboptimal for the cultivation of *A. brassicae*. Moreover, emphasised that CDA is a synthetic and chemically defined medium, lacking complex organic nutrients like various amino acids and vitamins required for the growth of this necrotrophic fungus. Further, also observed poor mycelial development and reduced sporulation of *A. brassicae* isolates on CDA compared to nutrient-rich media such as Potato Dextrose Agar (PDA) or V_8 juice agar. Similarly, research indicates that *Alternaria* species exhibited more vigorous colony growth and sporulation on media containing plant-based extracts, highlighting the importance of natural, complex substrates for in vitro propagation (35). Earlier, the reduced hyphal growth and pigment production of *Alternaria* spp. on CDA were noticed, which is attributed to the mediums' lack of stimulatory co-factors or secondary metabolites that may be present in natural substrates (36).

The reduced growth on CDA may also be ascribed to the funguss' ecological niche. Since *A. brassicae* typically colonizes senescent or stressed plant tissues, its physiological requirements may be more closely in line with the nutrient composition of decaying host material, which is not adequately replicated in chemically defined media like CDA. Thus, the consistent observation has been reported that limited growth on CDA across multiple studies emphasizes the critical role of media for fungal cultivation. For precise morphological and pathogenicity assessments, the use of complex or semi-natural media (e.g., PDA, V_8 agar, malt extract agar) is more useful, as they provide a more conducive environment for fungal metabolism, colony differentiation and sporulation. Moreover, such media facilitate more reliable comparisons among isolates in studies of pathogenic variability, fungicide sensitivity or morphological diversity (37).

Significant morphological variability has been reported among *A. brassicae* isolates, including characteristics like colour, texture, margin structure and zonation patterns. These variations are significantly influenced by environmental factors and growth media and may reflect underlying genetic diversity and adaptive potential of the pathogen (38). Research also indicates that colony colour in *A. brassicae* varied from light brown to black, with textures varying from compact to fluffy and margins from smooth to wavy (39, 40). Such differences not only assist in isolating differentiation but also have potential implications for pathogen virulence and host-pathogen interactions.

Similar findings were corroborated by earlier and more recent studies documenting a wide range of morphological traits across *A. brassicae* isolates. For instance, the notable variability in colony growth, surface topography, diversity in pigmentation, margin structure and sporulation behaviour was noticed (41–46). These morphological traits, often influenced by the culture medium and incubation conditions, are critical for understanding the pathogens' ecology, adaptability and potential aggressiveness on different *Brassica* hosts. Collectively, the morphological diversity observed among *A. brassicae* isolates reinforces the need for comprehensive characterisation approaches combining cultural, morphological and molecular data to accurately assess pathogen variability and its epidemiological implications.

Conclusion

The observed morphological and cultural variability among *A. brassicae* isolates is indicative of substantial genetic heterogeneity, governed by environmental conditions and host-associated factors. This phenotypic diversity emphasised the adaptive potential of the pathogen and has direct implications for its epidemiology and virulence across different agro-ecological zones of India. In the subtropical regions of northern India, where *Alternaria* blight is a major constraint to Brassica cultivation, knowing such variability is critical for the development of effective and region-specific disease management strategies at the farmer field as well as government-integrated disease management policy. The preferential growth of *A. brassicae* on potato dextrose agar and host leaf extract agar highlights the importance of nutrient-rich and biologically relevant media for accurate cultural characterisation. Host leaf extract agar facilitated substantial growth, likely due to its simulation of host-derived biochemical signals. Findings suggested that poor growth on synthetic media, such as czapek-dox agar, is due to inorganic nutrient profiles that are inadequate for supporting the physiological requirements of this necrotrophic pathogen.

Collectively, the findings emphasise the need for strategic selection of culture media in laboratory-based studies, particularly for fungal characterisation, pathogenicity assessment and variability screening. This study provides a foundational framework for enhancing diagnostic accuracy, guiding resistance breeding efforts and advancing our understanding of host–pathogen interactions in the Brassica.

Acknowledgements

The authors express their sincere gratitude to professors of the Department of Plant Pathology, Acharya Narendra Deva University of Agriculture and Technology (NDUAT), Kumarganj, Ayodhya- 224 229 (U.P.) India for its invaluable support and contributions to this research project.

Authors' contributions

HKS and MKM were responsible for the study design. DS and SP conducted the data arrangement. AK, VY, DSM and PY for manuscript preparation. AS, AY and SK edited the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no conflicts of interest to declare.

Ethical issues: None

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