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Variation in *Alternaria brassicae* Population Causing Alternaria Blight of Rapeseed and Mustard in Assam

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A B S T R A C T

Forty isolates of *Alternaria brassicae* extracted from the leaves and pods of rapeseeds and mustard collected from all the agro-climatic zones of Assam were studied for variability. The average conidial length and breadth of the isolates varied from 34.99µm to 46.36µm and 5.16µm to 9.72µm; and beak length from 4.01µmto 18.00µm. Numbers of horizontal septa and longitudinal septa ranged from 4.95 to 8.56 and 0.00 to 0.48, respectively. Four distinct colony colours - whitish gray; dark and brownish gray; gray, and greenish were identified. The isolates were compressed, slightly compressed, fluffy and slightly fluffy. Shapes of the colonies were either circular or irregular. The growth pattern was slow (0-40mm), medium (41-60mm) and fast (61-90mm). Molecular characterization revealed highest similarity (0.435) between Dhemajiand Darang isolates. Maximum dissimilarity (0.00) was observed between Jorhat isolate and Dhubri isolate, and between Sivsagar and Dhubri. The dendrogrambased on similarity index generated four clusters with 7, 15, 11and 7 isolates.

Introduction

India is said to be the paradise for oilseed crops being fourth largest oilseed producing country in the world harvesting about 25 million tons of oilseeds, and rapeseed and mustard alone contributed 32.00 per cent of total oilseed (Jha *et al.,* 2012). In 2013-14, it produced 74.90 lakh tones with a yield of 1147kg/ha (ADS, 2013). Sharma and Pandey (2013) reported that despite considerable production of oilseed, a wide gap exists between the potential yield and the yield realized at the farmers' field because of incidence of *Alternaria* blight. Researchers have reported yield loss due to *Alternaria* ranging from 36.88 % to 70.00 % (Kolte, 1985; Saharan, 1992; Kolte, 2002; Meena *et al.*, 2010; Bal and Kumar, 2014and Kumar *et al.*, 2014).

Morphological, cultural and genetic variability, together or separately, within the isolates of *Alternaria* spp. in different regions of India as well as in the world have been reported by several authors (Meena *et al.*,

1972; Gherbawy, 2005; Goyal *et al.,* 2011; Kaur *et al.,* 2015; Saha *et al.,* 2016; Aeneja *et.al.,* 2014; Bind *et al.,* 2014 and Selvamani, 2014).The importance of pathological as well as genetic level study has been highlighted for efficient management of the disease by Milgroom and Fry, 1997 and Aneja and Agnihotri, 2013.

In Assam, though large scale survey has not been carried out on the total loss caused by the *Alternaria brassicae* but reports from farmers' field about the disease is alarming requiring a full scale study to understand the variability of *Alternaria brassicae* to help develop management strategies.

Materials and Methods

Collection of the samples from different agro-climatic zones of Assam

Infected leaves and pods of rapeseed and mustard having the typical symptoms of *Alternaria* blight were collected from different agro-climatic zones of Assam (Table 1) and brought to the laboratory of Department of Plant Pathology, Assam Agricultural University, Jorhat within seven days of collection.

Fungal isolation

Forty isolates were isolated from the infected leaves and pods of rapeseed and mustard. The spots were first washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 4 per cent sodium hypochloride (NaOCl) solution for 1 minute, followed by washings with sterilized water 3-4 times. Surface sterilized leaf spot pieces were then aseptically transferred to 9cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 27±1°C for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot were aseptically transferred into another Petri plate containing PDA medium, where it was grown for another 15 days at 27±1°C in BOD incubator. In Petri plates, the diseased portion of the sample were cut and a small portion of the infected part was transferred to a culture plate containing PDA medium and kept under constant observation for the growth. After 5 days of incubation, the organisms were sub-cultured for purification. Slants of each culture were prepared from purified culture and were used further for morphological, cultural and molecular characterization of the fungus.

Morphological variation

Ocular micrometer was calibrated and by use of micrometry (Meena *et al.,* 2005), morphological variability among the 40 isolates of *A. brassicae* and other *Alternaria* spp. was studied in 2012-13 and 2013-14.

Cultural variation

Cultural characteristics of each isolates such as colony color characteristics as well as growth behavior such as morphological features and shape (circular/ irregular) was observed on PDA media and incubated in BOD incubator at 25°C temperature and 100 per cent relative humidity in order to detect the variation among the isolates. The colors of the cultures were taken in accordance with the help of the color chart of R.H.S (The Royal Horticultural Society, London). In this experiment, radial growth reading was taken at 15th day of inoculation of the culture on the Petri plates.

Molecular characterization

Isolation of genomic DNA

The forty isolates of *Alternaria* species were grown on PDA media for 14 days at 27±1°C.

Fungal mat was grinded in extraction buffer or lysis buffer and then total genomic DNA was isolated. The total genomic DNA was isolated by adopting CTAB method (Doyle and Doyle, 1990). The genomic DNA isolated from the isolates was checked for quality and quantity with sprectrophotometer and agarose gel electrophoresis.

RAPD analysis

Total 10 RAPD PCR primers were used starting from series OPD1, OPD2 to OPD 10 from Operon Technologies, USA which successfully amplified the DNAs and are depicted in Table 2.

The polymerase chain reaction (PCR) was carried out in Gene Amp 9700 thermal cycler PCR machine. The PCR master mix was prepared with 1X *Taq*polymerase buffer, 1.8 mM $MgCl_2$, 0.4 mMdNTPs, 0.4 pM primers and 1.5 U of *Taq*polymerase. Thereafter, 20 µl of master mix was added with 5 µl DNA in PCR tubes. Each PCR amplification reaction was preceded by an initial denaturation at 94°C for 4 min followed by forty PCR amplification cycles by denaturation at 94°C for 1 min, annealing at 37°C for 1 min and extension at 72°C for 1 min and then followed by final extension at 72°C for 10 min. The amplified products were separated by electrophoresis in 1.5% (w/v) agarose (Genei, Bangalore) gel with 1X TBE buffer, stained with ethidium bromide (0.5 µg/ml) at 90 V for 3.0 to 3.5 h and photographed using gel documentation system (Bio Rad, USA, model). The sizes of the amplification product were estimated using 1 kb ladder (Fermentas Life Sciences, Canada). Each PCR was repeated thrice and only sharp, reproducible amplicons were considered for fingerprint analysis of the isolates. Gel images were scored manually and recorded on the present/absent matrix data. These data were used as an input file for calculating similarity index using software NTSYS PC 2.02i (Rolf, 1997). A dendrogram was derived from the distance matrix by UPGMA (Sneath and Sokal, 1973).

Results and Discussion

Morphometric variation

Morphometric variation of forty isolates collected from six agro climatic zones depicted in Table 3 and Plate 1 showed high level of variability in spore morphology in respect to conidial length, width and number of both transverse and longitudinal septa.

In the total sample, average conidial length and breadth varied from 34.99µm (Is 7_Nal) to 46.36µm (Is 31_Siv) and 5.16µm (Is 18_Dhe and Is 28_Jor) to 9.72µm (Is 24_Nag) respectively. In case of beak length, the average length varied from 4.01µm (Is 9_Nal) to 18.00µm (Is 7_Nal). Horizontal septa showed higher degree of variation compared to longitudinal septa. Number of horizontal septa and longitudinal septa ranged from 4.95 (Is 7_Nal) to 8.56 (Is 14_Kok) and 0.00 (Is 18_Dhe) to 0.48 (Is 5_Dhu, Is 12_Bar, Is 17_Lak, Is 31_Siv) respectively.

Aneja *et al.*, 2014 reported high variation in spore size of *Alternaria brassicae* isolates with 37.7 to 257.6µm with beak sizes of 27.8 to 120.2µm. Similar findings were reported by Selvamani (2014), who collected forty isolates from different cauliflower, rapeseed and mustard growing locations in India and characterized for morphological variations.

All the isolates showed high level of variability in spore morphology in respect to conidial length, width and number of septa. The present findings were also in accordance with the findings of Goyal *et al*., (2011), who reported morphometric variation of thirteen *Alternaria brassicae* isolates of rapeseed and

mustard and found that average conidial length ranged from 31.2µm to 51.8µm, average conidial breadth ranged from 6.7µm to 9.6µm, average beak length ranged from 8.2µm to 19.2µm, transverse septa ranged between 4.8 to 7.2 numbers while longitudinal septa ranged from 0.0 to 0.4 numbers. A number of research workers (Saha *et al.*, 2016; Kaur *et al.*, 2015; Meena *et al.*, 2010 and Verma and Saharan, 1994) recorded morphological variations in *Alternaria* spp.

Cultural variation

Data presented in Table 4 and Plate 2, showed high level of variability among forty isolates collected from six Agro climatic zones. Variability was found *in vitro* in respect to mycelia growth rate, growth pattern or morphological features, colony shape and color.

The isolates showed varied colors in the PDA (Potato dextrose media). The colors were whitish gray, gray, brownish gray, dark gray and greenish.

Based on the findings, the total samples have been grouped into four sections accordingly. Isolates that showed whitish gray are grouped under Group I (Isolates Is 1_Kam, Is 7_Nal, Is 8_Nal, Is 15_Lak, Is 24_Nag, Is 33_Gol, Is 34_Gol, Is 38_NC, Is 39_Kar); dark gray and brownish gray are under Group II (Isolates Is 4_Dhu, Is 5_Dhu, Is 9_Nal, Is 10_Bar, Is 11_Bar, Is 12_Bar, Is 21_Dar); gray under group III (Isolates Is 2_Kam, Is 3_Kam, Is 6_Nal, Is 13_Kok, Is 14_Kok, Is 16_Lak, Is 17_Lak, Is 18_Dhe, Is 19_Dhe, Is 20_Dar, Is 22_Nag, Is 23_Nag, Is 25_Mor, Is 26_Mor, Is 27_Jor, Is 28_Jor, Is 30_Jor, Is 31_Siv, Is 32_Siv, Is 35_Dib, Is 36_Tin, Is 37_Kar) and greenish under group IV (Isolates Is 29_Jor and Is 40_Cac). Twenty three isolates showed gray color, nine showed whitish gray, four showed dark gray, two showed brown and two showed greenish color.

The isolates showed four types of morphological features i.e., compressed, slightly compressed, fluffy and slightly fluffy and most of the isolates showed fluffy type of morphological features. Thirty two showed fluffy, five compressed, two slightly compressed and one slightly fluffy. Twenty six isolates produced circular shaped colonies while fourteen colonies produced irregular shaped colonies.

In case of radial growth which was taken on 15th day of inoculation, the diameter ranged from 32.00 to 88.57mm. Highest growth was on Is 21_Dar (88.57mm), then comes Is 31_Siv with 88.25mm in diameter, lowest radial growth was in isolate Is 10_Bar (32.00mm), second lowest was in Is 30_Jor (32.05mm).The growth pattern was categorized into three types, *viz*., slow (0-40mm), medium (41-60mm) and fast (61-90mm). In slow growth five isolates (Is 4_Dhu, is 10_Bar, Is 11_Bar, Is 29_Jor and Is 30_Jor), medium three isolates (Is 12_Bar, Is 34_Gol and Is 35_Dib) and in fast growth remaining thirty two isolates were found.

The results were found to be very similar with that of Selvamani (2014), who characterized forty isolates of *Alternaria brassicae* from different cauliflower, cabbage and mustard growing in locations of India and found high variability *in vitro* in respect to mycelia growth, growth pattern and colour. Jha *et al.*, (2013) reported colony colors of *A. brassicae*as rather pale olive or gray or dark gray in color and having fluffy or compressed topography.

Meena *et al.*, (2012) found regular to irregular colony shape; cottony white, dark green to light brown mycelial growth among twenty three isolates of *A. brassicae* and further

grouped the colonies into four based on colony colors, *viz.*, Group 1 (white to pale gray), Group 2 (dark olive gray to iron gray colonies), Group 3 (gray to olive gray colonies) and Group 4 (lettuce green to olive green color). Kaur *et al.*, 2015 found the colonies brown, while and olevaceous green with smooth or wavy margins and thick velvety to sparse growth. Aneja *et al.*, 2014 and Saha *et al.*, 2016 also reported.

Table.1 *Alternaria brassicae* isolates collected from different pockets of six Agro-climatic Zones of Assam

Districts	Pockets	Isolates name	Latitude	Longitude
Lower Brahmaputra Valley Zone (LBVZ)				
Kamrup	Kahikuchi	Is 1_Kam	26.3588° N	91.1329° E
	Boko	Is 2_Kam	25.9778° N	91.2356° E
	Hajo	Is 3_Kam	26.2519° N	91.5257° E
Dhubri	Bilasipara	Is 4_Dhu	26.2300° N	90.2300° E
	Mancachar	Is 5_Dhu	25.5300° N	89.8700° E
Nalbari	Belshor	Is 6_Nal	26.3981° N	91.3638° E
	Goreshwar	Is 7_Nal	26.5400° N	91.7300° E
	Kamarkuchi	Is 8_Nal	26.0856° N	91.8537° E
	Tihu	Is 9_Nal	26.4749° N	91.2689° E
Barpeta	Pathshala	Is 10_Bar	26.4994° N	91.1793° E
	Bahari	Is 11_Bar	26.2546° N	91.1379° E
	Baradi	Is 12_Bar	26.3274° N	91.0507° E
Kokrajar	Gossaigaon	Is 13_Kok	26.4197° N	89.9842° E
	Salakati	Is 14_Kok	26.4933° N	90.3625° E
North Blank Plain Zone (NBPZ)				
Lakhimpur	Ghalimora	Is 15_Lak	26.4400° N	92.3456° E
	Dhokuwakhana	Is 16_Lak	27.2300° N	94.1000° E
	Narayanpur	Is 17_Lak	26.9964° N	93.8969° E
Dhemaji	Gogamukh	Is 18_Dhe	27.4303° N	94.3102° E
	Bordoloni	Is 19_Dhe	26.8022° N	93.5635° E
Darrang	Machkhowa	Is 20_Dar	26.1779° N	91.7374° E
	Norowathan	Is 21_Dar	27.2861^{o} N	94.4449° E
Central BrahmaputraValley zone (CBVZ)				
Nagaon	Shillongoni	Is 22_Nag	26.3503° N	92.6922° E
	Raha	Is 23_Nag	26.2327° N	92.5278° E
	Mikirgaon	Is 24_Nag	26.1407° N	92.6933° E
Morigaon	Mayong	Is 25_Mor	26.2589° N	92.0408° E
	Banmurigaon	Is 26_Mor	26.4800° N	90.5600° E
Upper Brahmaputra Valley zone (UBVZ)				
Jorhat	Titabor	Is 27_Jor	26.6000° N	94.2000° E
	Teok	Is 28_Jor	26.8130° N	94.4065° E
	AAU, ICR farm	Is 29_Jor	26.4400° N	94.0000° E
	Majuli	Is 30_Jor	26.9500° N	94.1667° E
Sivsagar	Dimow	Is 31_Siv	27.1268° N	94.7400° E
	Amguri	Is 32_Siv	26.5800° N	94.5230° E
Golaghat	Dergaon	Is 33_Gol	26.7000° N	93.9700° E
	Borpothar	Is 34_Gol	27.4728^{o} N	94.9119° E
Dibrugarh	Sarupathar	Is 35_Dib	26.1946° N	93.8629° E
Tinsukia	Doom Dooma	Is 36_Tin	27.5700° N	95.5700° E
Hill Zone (HZ)				
KarbiAnglong	Badarpur	Is 37_Kar	24.9000° N	92.6000° E
North Cachar Hill	Haflong	Is 38_NC	25.1800° N	93.0300° E
Barrak Valley Zone (BVZ)				
Karimgang	Diphu	Is 39_Kar	25.8300° N	25.8300° N
Cachar	Silchar	Is 40_Cac	24.8200° N	24.8200° N

Table.2 Primer code and sequence of fragments amplified by the RAPD marker

Primer	Sequence 5/ to 3/
OPD1	ACCGCGAAGG
OPD2	GGACCCAACC
OPD 3	GTCGCCGTCA
OPD 4	TCTGGTGAGG
OPD 5	TGAGCGGACA
OPD 6	ACCTGAACGG
OPD 7	TTGGCACGGG
OPD 8	GTGTGTCCCA
OPD 9	CTCTGGAGAC
OPD 10	GGTCTACACC

Table.3 Morphological variation of *Alternaria brassicae* isolates from different agro-climatic Zones of Assam

Isolate	Conidial length (µm)		Conidial breadth (µm)		Beak length (µm)		Septation (µm)			
	Average	Range	Average	Range	Average	Range	Transverse		Longitudinal	
							Average	Range	Average	Range
LBVZ										
Is 1_ Kam	35.62	35.00-38.50	6.63	6.00-7.50	10.76	9.50-15.50	5.05	4-6	0.42	0-2
Is 2_Kam	38.30	34.30-40.40	6.19	5.50-6.00	11.00	9.00-18.00	6.01	6-7	0.38	0-2
Is 3_Kam	38.22	35.00-40.80	6.25	6.00-7.20	10.50	8.00-15.50	5.01	5-6	0.24	0-1
Is 4_Dhu	41.18	30.00-50.00	8.73	7.20-12.00	13.52	9.50-20.00	6.93	5-7	0.41	0-2
Is 5_Dhu	40.90	30.00-45.00	8.93	7.00-11.00	8.44	4.80-12.00	6.97	5-7	0.48	0-4
Is 6_Nal	36.18	30.00-36.00	7.22	6.00-6.50	8.28	4.80-12.00	5.11	5-6	0.26	0-2
Is 7_Nal	34.99	30.00-35.00	5.85	6.00-6.50	18.00	9.00-20.00	4.95	4-5	0.41	0-2
Is 8_Nal	37.75	30.00-38.00	6.18	5.00-6.00	8.59	4.85-11.55	5.01	4-6	0.00	0-0
Is 9_Nal	37.60	30.00-40.00	6.22	5.00-6.00	8.55	4.55-12.00	5.12	4-5	0.16	0-1
Is 10_Bar	40.33	38.00-45.00	7.85	10.00-12.00	4.01	2.01-7.09	7.38	6-8	0.21	0-3
Is 11_Bar	36.36	34.00-37.50	5.33	5.00-7.54	10.66	9.53-15.45	5.29	4-6	0.45	0-1
Is 12_Bar	37.86	34.35-40.43	6.06	5.54-6.06	11.66	9.05-18.05	5.89	4-5	0.48	0-2
Is 13_Kok	38.18	35.05-45.80	6.35	6.50-7.20	11.60	8.00-15.60	5.40	6-7	0.28	0-1
Is 14_Kok	45.18	34.00-50.00	9.69	7.40-11.07	13.93	10.54-24.00	8.56	7-8	0.44	0-2
NBPZ										
Is 15_Lak	41.13	34.00-46.00	9.48	7.55-11.30	13.70	9.05-24.00	8.48	7-9	0.02	0-0
Is 16_Lak	36.66	30.00-36.00	6.00	6.00-4.50	8.70	4.90-12.50	5.93	5-7	0.41	0-1
Is 17_Lak	45.16	30.00-36.00	6.56	6.55-6.505	8.80	4.75-12.40	6.13	5-7	0.48	0-1
Is 18_Dhe	36.74	30.00-38.00	5.16	5.06-6.06	8.54	4.84-11.00	5.03	4-5	0.00	0-0
Is19_Dhe	37.33	32.05-40.05	6.51	5.05-6.05	4.02	2.00-5.05	5.88	5-6	0.20	0-1
Is 20_Dar	40.00	38.35-45.20	8.18	10.00-12.00	6.05	3.00 -9.05	7.42	6-7	0.21	0-3
Is 21_Dar	38.36	35.35-38.50	6.33	6.00-7.50	10.86	9.50-15.50	5.91	5-6	0.40	0-2
CBVZ										
Is 22_Nag	36.14	35.30-41.40	6.29	5.50-6.00	11.14	9.00-18.00	6.05	6-7	0.40	0-2
Is 23_Nag	40.04	38.00-43.80	6.32	6.00-7.20	10.66	8.00-15.50	5.67	5-6	0.21	0-1
Is 24_Nag	40.67	30.00-45.00	9.72	7.20-12.00	13.66	9.50-20.00	6.92	5-7	0.40	0-2
Is 25_Mor	40.11	30.55-46.00	9.66	7.00-11.00	13.51	9.00-20.00	6.60	5-7	0.45	0-3
Is 26_Mor	37.44	37.00-36.00	6.11	6.00-6.50	8.24	4.86-12.50	5.48	5-6	0.45	0-2
UBVZ										
Is 27_Jor	35.66	30.00-35.00	6.07	6.00-6.50	8.00	4.80-12.00	5.59	5-6	0.41	0-2
Is 28_Jor	35.85	30.00-38.00	5.16	5.00-6.00	8.00	4.80-11.00	5.28	5-6	0.45	0-3
Is 29_Jor	38.00	30.00-40.00	6.33	5.00-6.00	8.00	4.00 -10.00	5.18	5-6	0.06	0-1
Is 30_Jor	42.53	40.00-45.00	8.15	10.00-12.00	8.96	4.00 -11.05	6.04	6-7	0.21	0-3
Is 31_Siv	46.36	38.00- 47.08	6.37	6.00-7.50	10.75	9.70-15.50	5.23	5-6	0.48	0-2
Is 32_Siv	38.33	34.30-40.40	6.08	5.50-6.00	11.00	9.00-18.00	6.16	6-7	0.47	0-2
Is 33_Gol	37.55	35.00-40.80	6.25	6.00-7.20	10.55	8.00-15.50	5.49	4-6	0.23	0-1
Is 34_Gol	40.71	30.00-50.00	8.55	7.20-12.00	17.62	9.50-20.00	7.58	5-8	0.41	0-2
Is 35_Dib	38.58	30.00-45.00	8.15	7.00-11.00	13.29	9.00-20.00	7.05	5-8	0.13	0-0
Is 36_Tin	35.55	30.00-36.00	6.04	6.00-6.50	8.23	4.80-12.00	5.55	4-6	0.40	0-2
HZ										
Is 37_Kar	35.32	30.00-35.00	6.32	6.05-6.50	13.02	11.98-14.00	5.89	4-6	0.34	0-2
Is 38_NC	36.29	30.00-38.00	5.18	5.00-6.00	8.00	4.80-11.00	4.83	4-5	0.05	0-0
BVZ										
Is 39_Kar	38.04	30.00-40.00	6.74	5.00-6.00	10.01	6.00 -12.05	5.44	4-6	0.20	0-3
Is 40_Cac	45.55	38.00-46.00	8.52	11.00-12.00	7.01	5.05 -8.09	5.53	4-6	0.38	0-1
CD (5%)	**1.448**		**0.980**		**0.849**		**1.55**		**1.55**	

Table.4 Cultural variation of *Alternaria brassicae* isolates from different agro-climatic Zones of Assam

Isolate	Cultural characteristics				
	Colour on PDA plates	Morphological features	Shape (Circular/ irregular)	Radial Growth on 15th day of inoculation (mm)	Groups
LBVZ					
Is 1_ Kam	Whitish gray	Compressed	Circular	76.05	I
Is 2_Kam	Gray	Fluffy	Circular	75.95	III
Is 3_Kam	Gray	Fluffy	Circular	62.23	III
Is 4_Dhu	Dark gray	Compressed	Irregular	37.90	II
Is 5_Dhu	Dark gray	Compressed	Circular	75.05	II
Is 6_Nal	Gray	Slightly compressed	Circular	72.00	III
Is 7_Nal	Whitish gray	Fluffy	Circular	80.03	I
Is 8_Nal	Whitish gray	Fluffy	Circular	81.00	I
Is 9_Nal	Dark Gray	Fluffy	Circular	80.00	II
Is 10_Bar	Brownish gray	Fluffy	Irregular	32.00	II
Is 11_Bar	Brownish gray	Fluffy	Irregular	34.76	II
Is 12_Bar	Gray	Fluffy	Irregular	42.00	II
Is 13_Kok	Gray	Fluffy	Circular	76.06	III
Is 14_Kok	Gray	Fluffy	Irregular	72.95	III
NBPZ					
Is 15_Lak	Whitish gray	Fluffy	Circular	79.09	I
Is 16_Lak	Gray	Fluffy	Irregular	65.05	III
Is 17_Lak	Gray	Fluffy	Circular	80.32	III
Is 18_Dhe	Gray	Fluffy	Circular	81.44	III
Is19_Dhe	Gray	Fluffy	Irregular	72.07	III
Is 20_Dar	Gray	Fluffy	Irregular	62.06	III
Is 21_Dar	Dark gray	Slightly compressed	Circular	88.57	II
CBVZ					
Is 22_Nag	Gray	Fluffy	Irregular	77.54	III
Is 23_Nag	Gray	Fluffy	Circular	87.77	III
Is 24_Nag	Whitish gray	Fluffy	Circular	75.55	I
Is 25_Mor	Gray	Slightly fluffy	Circular	87.79	III
Is 26_Mor	Gray	Fluffy	Circular	72.00	III
UPVZ					
Is 27_Jor	Gray	Compressed	Circular	67.05	III
Is 28_Jor	Gray	Fluffy	Circular	66.09	III
Is 29_Jor	Greenish	Fluffy	Circular	35.05	IV
Is 30_Jor	Gray	Fluffy	Irregular	32.05	III
Is 31_Siv	Gray	Fluffy	Circular	88.25	III
Is 32_Siv	Gray	Fluffy	Irregular	80.75	III
Is 33_Gol	Whitish gray	Fluffy	Circular	80.77	I
Is 34_Gol	Whitish gray	Fluffy	Irregular	55.59	I
Is 35_Dib	Gray	Fluffy	Irregular	57.44	III
HZ					
Is 36_Tin	Gray	Compressed	Circular	88.05	III
Is 37_Kar	Gray	Fluffy	Circular	81.16	III
Is 38_NC	Whitish Gray	Fluffy	Irregular	65.55	I
BVZ					
Is 39_Kar	Whitish Gray	Fluffy	Circular	79.22	I
Is 40_Cac	Greenish	Fluffy	Circular	72.55	IV

Table.5 Primer-wise score of PCR amplification products scored in the 40 isolates of *Alternaria brassiace*

Primer	Number of PCR amplification fragments generated	
	Monomorphic bands	Polymorphic bands
OPD1	1	7
OPD2	0	8
OPD3	2	5
OPD4	1	5
OPD5	0	6
OPD6	0	7
OPD7	0	8
OPD8	2	5
OPD9	1	5
OPD10	2	6

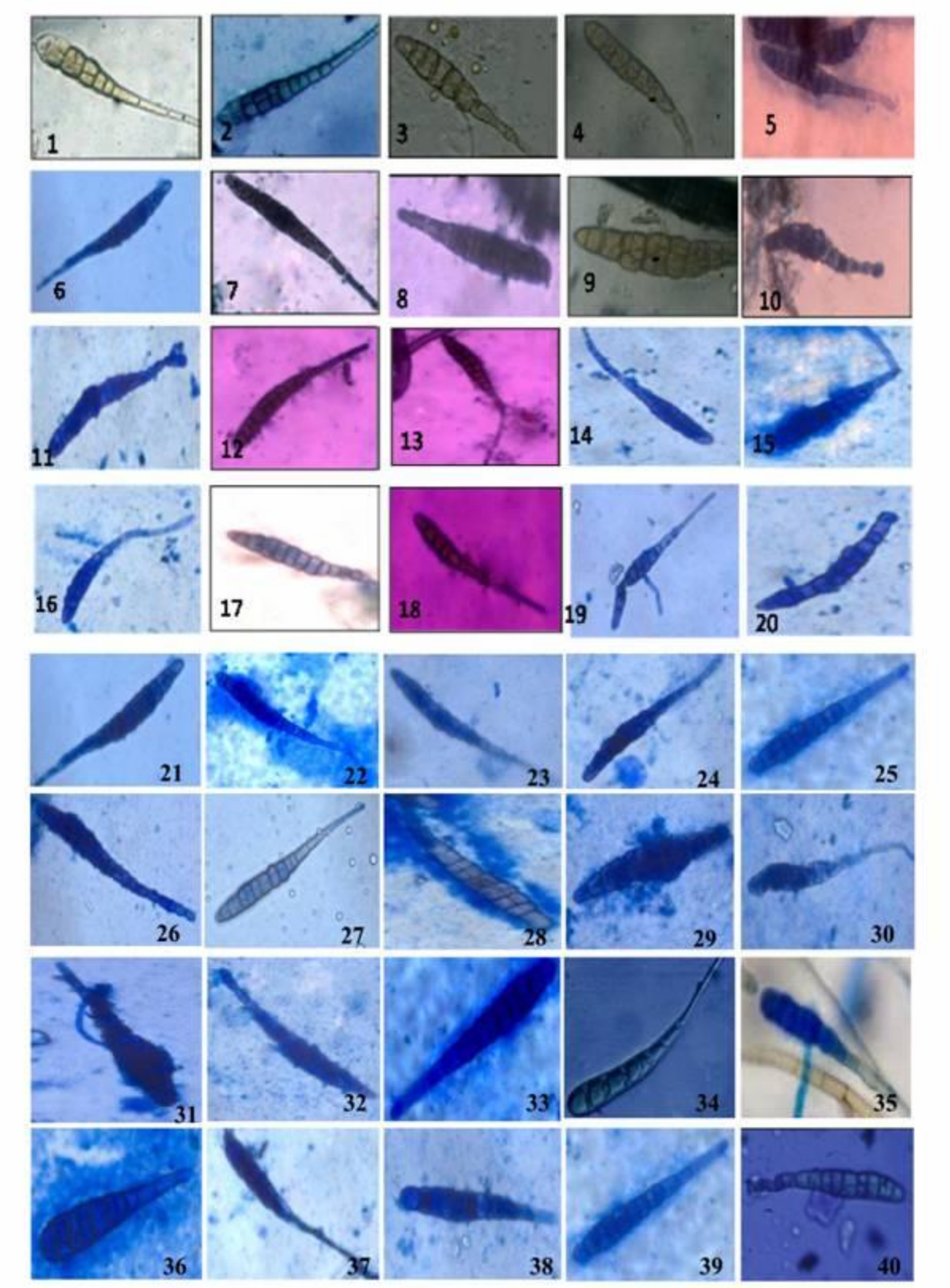


Plate 1: Conidial structures of *Alternaria brassicae* (1-40) under Phase Contrast microscope (Maginification 40X)

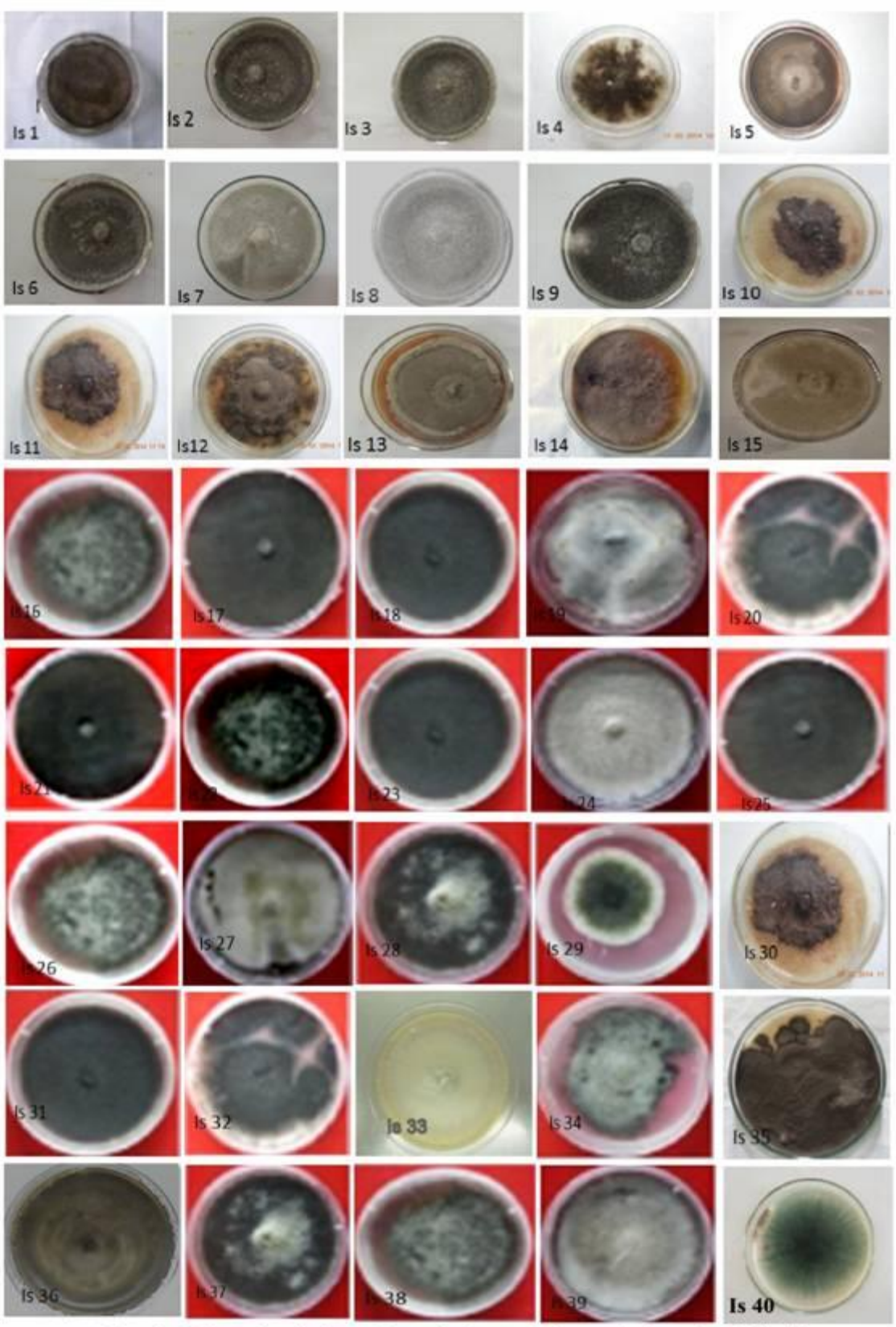
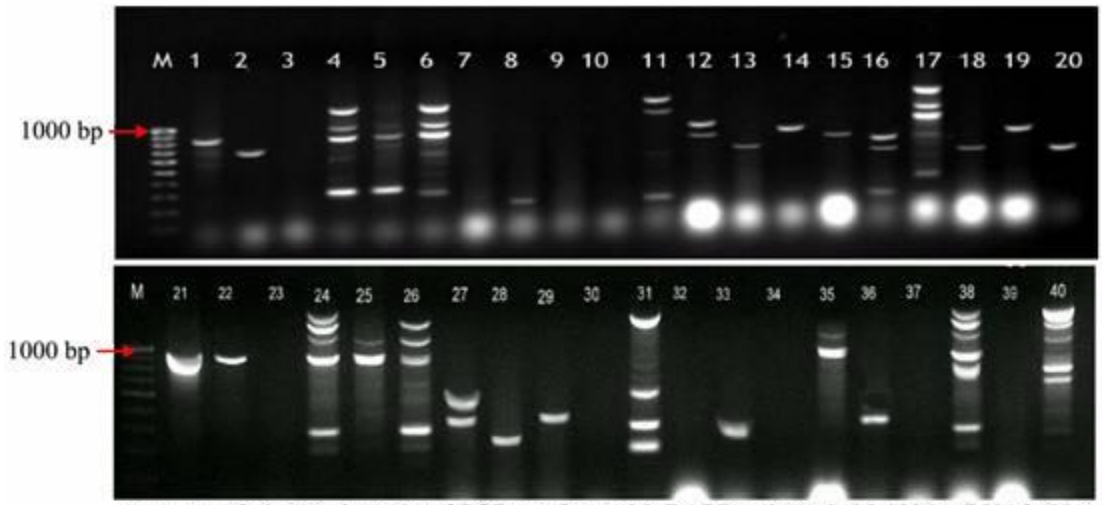
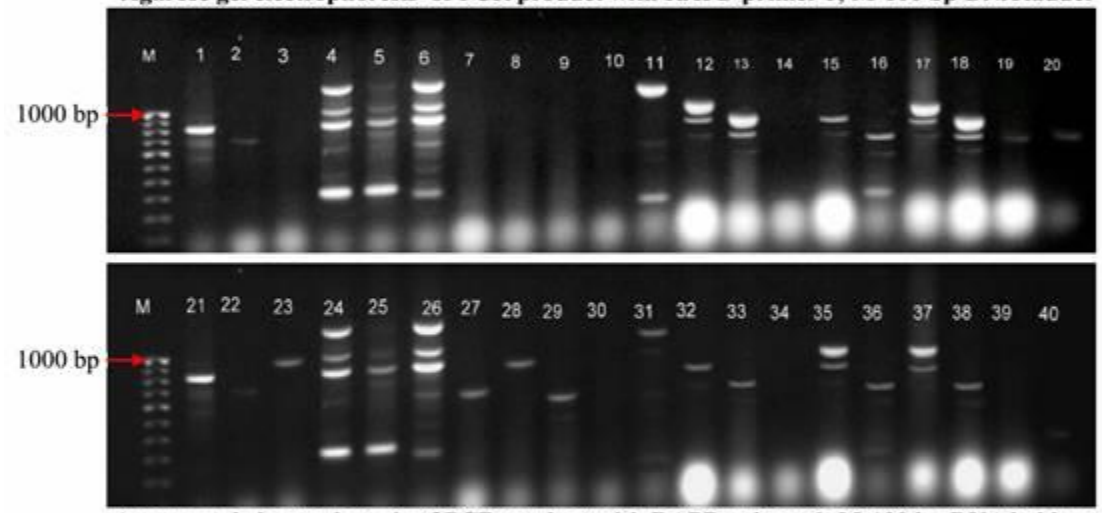


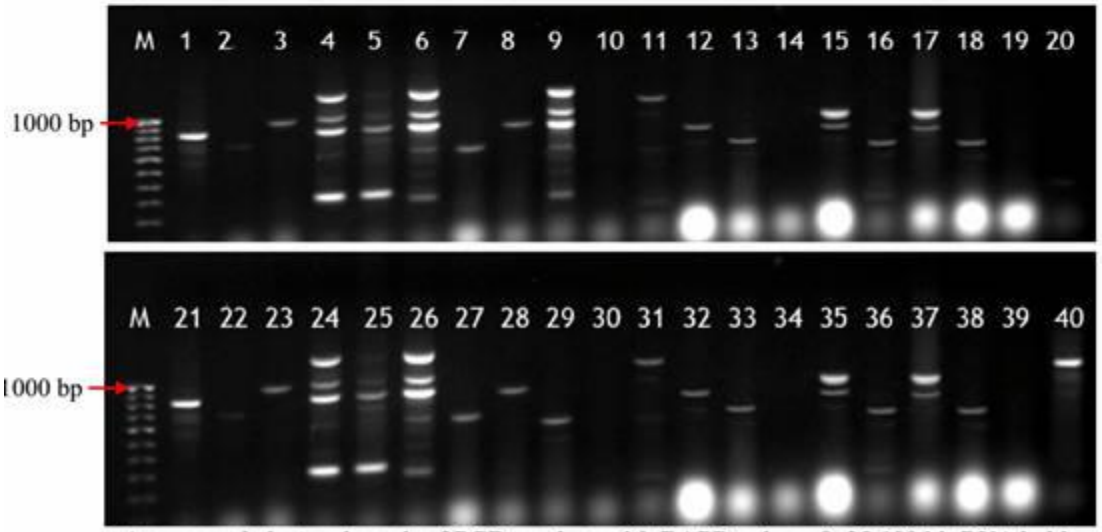
Plate 2: Cultural variation of forty isolates of *Alternaria brassicae* (Is1-40)



Agarose gel electrophoresis of PCR product with RAPD primer 1; M-100 bp DNA ladder



Agarose gel electrophoresis of PCR product with RAPD primer 6; M-100 bp DNA ladder



Agarose gel electrophoresis of PCR product with RAPD primer 9; M-100 bb DNA ladder

Plate 3. Molecular characterization

Molecular characterization

The authenticity of variations among the various isolates of *A. brassicae* can be obtained only after confirming their genetic variations. It is considered as powerful tools to analyze genetic relationships and diversity. Forty isolates of *A. brassicae* of rapeseed and mustard which were subjected for molecular characterization using ten RAPD markers (OPD1 to OPD 10) generated good number of polymorphic bands and the polymorphism was clearly distributed as shown in Table 5. Good quality genetic profiling was obtained. A total of 71 RAPD loci were amplified from different isolates. Most of the PCR products were in size range of 650–1000 bp. Out of the 71 bands scored 62 (87.32 %) were found to be polymorphic and 9 (12.67%) were found to be monomorphic in nature. The frequencies of polymorphic bands obtained varied from primer to primer. Wide genetic variation between isolates of the species was evident from the high number of polymorphic marker and unique bands, even though small number of isolates available.

Jaccard's coefficient of similarity among the isolates was calculated. Maximum similarity was found in between Dhemaji isolate (Is 18_Dhe) and Darang isolate (Is 20_Dar) with coefficient of similarity (0.435). Next to this, 0.400 coefficient of similarity was found between Darang (Is 21_Dar) and Dhemaji (Is 19_Dhe) and also between Darang (Is 21_Dar) and Darang (Is 20_Dar). All the above four isolates were extracted from samples collected from single agro-climatic zone-NBPZ. The isolates, Is 18_Dhe and Is 20_Dar are gray and fluffy and shared similarities in morphological parameters.

Maximum dissimilarity was observed between Jorhat isolate (Is29_Jor) and Dhubri isolate (Is 4_Dhu) and between Sivsagar (Is 32_Siv) and Dhubri (Is 5_Dhu) i.e 0.00. By comparing the amplification profile with 30 bands, it has been found that there was no sharing of common band between each pairs of the isolates (Is 29_Jor, Is 4_Dhu and Is 32_Siv and Is 5_Dhu). However, Jaccard's Coefficient of similarity takes into account sharing of the amplified band that may be the reason for getting 0.00 per cent similarity. Use of more numbers of markers might have resolved the problem of 0.00 per cent similarity. Next to zero per cent, least genetic similarity (0.038) was found between Jorhat (Is 30_Jor) and Kamrup (Is 1_Kam) and also between Jorhat (Is 30_Jor) and Barpeta (Is 11_Bar) isolates. The Is29_Jor isolate was greenish, fluffy and circular in shape while the Dhubri isolate (Is 4_Dhu) was dark gray, compressed and irregular shaped. In terms of morphological character, average conidial length, breadth, beak length and septation were much less in Is29_Jor isolate compared to Is 4_Dhu. Similar trend was observed between Dhubriisolate (Is 5_Dhu) and Sivsagarisolate (Is 32_Siv).

The dendrogram (Fig. 1) generated with the similarity data was grouped into four clusters. Cluster I consist of 7 isolates (Is 1_Kam, Is 7_Nal, Is 8_Nal, Is 4_Dhu, Is 14_Kok, Is 33_Gol and Is 40_Cac). Cluster 1 isolates were mostly concentrated in LBVZ with whitish gray and fluffy growth. Cluster II isolates (Is 2_Kam, Is 5_Dhu, Is 30_Jor, Is 31_Siv, Is 6_Nal, Is 38_NC, Is 24_Nag, Is 34_Gol, Is 39_Kar, Is 9_Nal, Is 11_Bar, Is 12_Bar, Is 13_Kok, Is 16_Lak and Is 25_Mor) spread over all the geographical locations with maximum concentration in LBVZ and were mostly gray, fluffy and circular shaped. Cluster III consists of 11 isolates (Is 10 Bar, Is 26_Mor, Is 27_Jor, Is 28_Jor, Is 17_Lak, Is 18_Dhe, Is 20_Dar, Is 19_Dhe, Is 21_Dar, Is 35_Dib and Is 36_Tin) and were concentrated in NBPZ and UBVZ. They exhibited mostly gray, fluffy and circular character. The Cluster IV consists of

7 isolates (Is 15_Lak, Is 32_Siv, Is 37_Kar, Is 22_Nag, Is 23_Nag, Is 3_Kam and Is 29_Jor) spread over almost all the zones and were fluffy. The information reveals genetic and cultural and morphological variation in the isolates across the state.

The present findings are supportive of Aneja *et al.*, 2014 who reported high level of genetic diversity (57% to 78%) amongst *A. brassicae* isolates as revealed by RAPD banding profile. The analysis of similarity matrix data also revealed high level of diversity (82.83%) among all the isolates from different regions of India (Aneja*et al.*, 2014).

The study confirms Cultural, morphological and genetical variations among the *Alternaria brassicae* isolates across different agro-climatic zones of Assam. The isolates with different genetical characterization did not express similar morphological and cultural characteristics. The results provide useful information for breeding programs, epidemiological studies as well as improved disease management strategies.

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