The Pharma Innovation Journal 2023; 12(4): 2327-2336

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 2327-2336

www.thepharmajournal.com
Received: 16-02-2023
Accepted: 19-03-2023

Arghya Banerjee
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Partha Sarathi Nath
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Corresponding Author:
Arghya Banerjee
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Cultural, morphological and genetic variability of *Exserohilum turcicum* (Pass.) Leonard and Suggs. in West Bengal

Arghya Banerjee and Partha Sarathi Nath

Abstract
Among the foliar diseases of maize, the turcicum leaf blight (TLB) also called as Northern corn leaf blight caused by *Exserohilum turcicum* (Pass) Leonard and Suggs. (syn. *Helminthosporium turcicum* Pass.) is having worldwide importance. Turcicum leaf blight is one of the most important foliar fungal diseases affecting photosynthesis with severe reduction in grain yield of more than 50 per cent. The eight (8) *Exserohilum* isolates (Et1 – Et8) collected from different districts of West Bengal *viz.*, Nadia, Bardhaman, Bankura, Birbhum, Murshidabad, Purulia, Purba Medinipur and Coochbehar districts during Kharif 2017-18 to assess the disease severity and to study variability of the pathogen. The eight single spore-cultures of *E. turcicum* isolates (Et) exhibited cultural variability in respect of colony colour, colony growth, sporulation and pigmentation on best suited four media such as PDA, MLEA, PCA and PDA+Yeast at different temperature ranges. Colony colour varied from grey to black in colour depending upon the isolates and growth medium. The isolate Et4 (Birbhum), Et6 (Purulia), Et7 (Purba Medinipur) and Et8 (sorghum, Coochbehar) were under the group of profuse to moderate growth was exhibited in the isolates Et1 (Nadia) and Et2 (Bardhaman) while poor restricted growth was observed in the Et3 (Bankura) and Et5 (Murshidabad) isolates. The isolates Et1 (Nadia), Et4 (Birbhum), Et6 (Purulia), Et7 (Purba Medinipur) produced excellent sporulation while the isolates Et2 (Bardhaman), Et3 (Bankura) exhibited good sporulation and showed the similarity with sorghum isolate Et8. The isolates Et1 recorded the maximum (117.26 µm) conidial length and minimum (82.50 µm) conidial length was recorded by the isolate Et5. The isolates that were identical for virulence were dissimilar with RAPD markers. The most virulent Birbhum and Purulia isolates were grouped in different sub groups.

Keywords: Maize, turcicum leaf blight, variability, RAPD, West Bengal

Introduction
Maize (*Zea mays* L.) is an important coarse cereal and is the third major crop in India after rice and wheat. Maize is native of Mexico and Central America by origin. Norman E. Borlaug believed that maize has the highest yield potential among cereals. In the last two decades there was a revolution in rice and wheat and probably the next few decades will be known as era of maize (Anon., 2010) [1]. In India, it is grown over an area of 8.69 mha with a total production of about 21.81 mt in major maize growing states like Karnataka, Andhra Pradesh, Maharashtra, Uttar Pradesh, Bihar, West Bengal, Rajasthan, Madhya Pradesh and Punjab together contribute 60 per cent of area and 70 per cent of maize production in India. The average maize yield in India is 25.09 q/ha, which is much lower than most of the maize growing countries of the world (Anon., 2016) [2]. About 61 diseases have been reported in India which affects the maize crop (Payak and Sharma, 1985) [15]. Based on the research efforts for the last few years under the All India Coordinated Maize Improvement Project, 16 out of 61 diseases adversely affecting this crop have been identified as major diseases. With the introduction of high yielding hybrids both indigenous, exotic and as well as use of fertilizers, there has been a tremendous increase in the area and production. But at the same time, it is prone to several foliar and stalk rots, downy mildews and ear rots (Payak *et al*., 1973 and Payak and Sharma, 1985) [14, 15]. Foliar diseases of maize are arguably the primary biotic constraints to maize yields worldwide and the prevalence of these foliar diseases varies depending on the region or season (Smith, 1999) [19]. Among the foliar diseases of maize, the turcicum leaf blight (TLB) also called as Northern corn leaf blight caused by *Exserohilum turcicum* (Pass) Leonard and Suggs. (syn. *Helminthosporium turcicum* Pass.) is having worldwide importance. Turcicum leaf blight is one of the most important foliar fungal diseases affecting photosynthesis with severe reduction in grain yield of more than 50 per cent

(Raymundo and Hooker, 1981 and Perkins and Pederson, 1987) [17, 16]. In the present study, focus was made towards cultural, morphological and genetic variability of *Exserohilum turcicum* (Pass.) Leonard and Suggs. in West Bengal condition.

Materials and Methods

Disease survey and collection of Turcicum leaf blight (TLB) diseased samples

Field roving surveys were conducted at different location of eight districts of West Bengal (Nadia, Burdwan, Bankura, Birbhum, Purulia, Murshidabad, Purba Medinipur and Coochbehar) for collection of Turcicum leaf blight infected diseased samples of maize crop (Table 1). The fresh leaf samples exhibiting typical symptoms of the disease usually with greyish green spindle shaped lesions having sporulation were brought to laboratory for microscopic examination and isolation of the causal pathogen/fungus and for future experimental works. Polypropylene bags were used for sample collection.

Table 1: Description of site of collection for the isolation of *Exserohilum turcicum* isolates

Serial No.	Isolate name	Plant parts	Location of sample collection
1	Et1	Leaf (Maize)	Kalyani, Nadia
2	Et2	Leaf (Maize)	Aushgram-II, Bardhaman
3	Et3	Leaf (Maize)	Sampur, Bankura
4	Et4	Leaf (Maize)	Shekhampur, Birbhum
5	Et5	Leaf (Maize)	Kandi, Murshidabad
6	Et6	Leaf (Maize)	Kashipur, Purulia
7	Et7	Leaf (Maize)	Nandakumar, Purba Medinipur
8	Et8	Leaf (Sorghum)	Pundibari, Coochbehar

Isolation, Purification and Identification of the pathogen:

The infected leaves of maize showing typical symptoms were collected from different locations of West Bengal. The standard tissue isolation procedures were followed to isolate the pathogen. The infected portion was thoroughly washed. In tap water and separately cut into small pieces of 0.5 cm in size, showing half healthy and half diseased area, with the help of a sterilized blade. These pieces were then surface disinfected with 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds, followed by 3-4 changes/ washing in sterilized distilled water. The surface disinfected pieces were aseptically transferred separately to the Petri plates containing solidified PDA or Water Agar medium and then incubated at 25 ± 1^0C. After 5 days of incubation, the growing mycelium from the margin of apparently distinct colonies were subcultured on fresh Petri plates containing suitable medium. The culture of the fungus, thus obtained were purified by single spore isolation (Ho and Ko, 1997) [10] and maintained on PDA slants to keep the culture viable. Such slants were preserved in refrigerator at 5 ºC and sub-cultured once in two months' interval, and kept for further studies. The fungal culture was examined under compound microscope for the mycelial and spore characteristics. The confirmation and specific identification of the culture was done on the basis of the characters described by Muiru *et al*. (2008) [13], Harlapur and Kulakarni (2009) [9].

Studies on growth, morphology and sporulation of pathogenic isolates on different laboratory culture media

Twelve culture media *viz*., Potato Dextrose Agar (PDA), V-8 juice agar, Czapek's Dox Agar (CDA), Oat Meal Agar (OMA), Peptone Salt Agar (PSA), Maize leaf extract agar (MLEA), Potato carrot agar (PCA), Yeast Extract Agar (YEA), PDA+ Yeast, Maize Leaf Extract Dextrose Agar (MDA), Maize Leaf Extract Sucrose Agar (MSA) and Maize Leaf Extract Oatmeal Agar (MOA) were used to find out the preferred medium for the radial growth, sporulation and study of colony morphology of Et isolates. Spore length and breadth was measured using binocular microscope through Axiom Vision software.

Effect of different pH on mycelial growth

To study the effect of pH on radial growth characteristics, four different levels of pH viz. 5.0, 6.0, 7.0, and 8.0 were maintained in PDA media. The different pH levels were adjusted with HCI and NaOH as per requirement. After inoculation, the Petri dishes were incubated at 25 ± 1^0C. Three dishes or replications were used for each treatment.

Determination of intensity of conidial sporulation

Spore intensity on different media was recorded 15 days after incubation. Twenty mycelial discs of 5 mm diameter were cut from equal distance in the Petriplates from medium using sterilized cork borer and five such discs were placed per vial, containing 1ml water plus lactophenol (9:1) suspension, with the help of sterilized inoculation needle. The vials were vigorously shaken and discs scrapped to get release of the spores in suspension. The suspensions were used to determine the intensity of sporulation with the help of a haemocytometer.

Morphological studies

To study the morphological characters, 5 mm mycelial disc of actively growing *E. turcicum* isolates collected from >15 days old culture isolated from different locations were transferred on Petri plates containing sporulating medium and incubated at 25 ± 1 ºC for 15 days. Morphological characters such as conidial length and breadth and presence or absence of conidial hilum on conidiophores, septations, shape of conidia were studied. Measurement of total length and breadth of conidia were done by micrometric measurement with Axiom Vision Software. Fifty conidia from each isolates were measured after 15 days of incubation.

Characterization of isolates by RAPD

The molecular variability among eight isolates of *E. turcicum* was studied using random amplified polymorphic DNA (RAPD). Standard protocols were used for the isolation of DNA and RAPD analysis (Williams *et al*., 1990) [21].

a. Fungal cultures

Mycelial discs (5 mm diameter) from periphery of an actively growing 12 days old culture on PDA were inoculated into 250 ml conical flask containing 100 ml of sterile Potato Dextrose Broth. The flasks were incubated for 10 days at 25 +1 ºC. The resultant growth of the mycelial mat was harvested and the excess moisture was completely removed using sterile blotting paper. The mat was used for DNA extractions.

b. Isolation of genomic DNA

Ten-day old culture of all the isolates of the pathogen was used for DNA isolation. The mycelial mat was taken out from PDB and blot dried after thorough washing with sterile distilled water and 500 mg of mycelium was taken with

respect to each of the isolate for isolation of DNA. The mat was grinded into fine powder in a small pestle and mortar by adding liquid nitrogen. Immediately the powder was poured in an eppendorf tube containing 1 ml of CTAB buffer and mixed thoroughly. Then the samples were incubated in a water bath at 65 ºC for 30 minutes and cooled to room temperature. One ml of chloroform and isoamyl alcohol (Chloroform: isoamyl alcohol = 24:1) was added to each tube, mixed and centrifuged at 3,500 rpm for 20 minutes in high speed refrigerated centrifuge (Eppendorf, USA). The supernatant was collected carefully into another eppendorf tube by 200 µl micropipette and re-extracted with 1.0 ml of chloroform: isoamyl alcohol (24: 1).

Finally, the aqueous layer was collected in another eppendorf tube and 2/3 volume of isopropanol was added to it and the content was mixed by tilting the tubes gently and kept at -20 ºC for 30 minutes to allow the DNA to precipitate. After that a spin at full speed (10,000 rpm) was given for 3 minutes in a centrifuge and the DNA pellet was washed with 70 per cent ethyl alcohol 3 times to remove the salts and other metabolites and air dried. The DNA pellet was suspended in 200 µl of T10 E1 buffer and stored at -20 ºC for further use. The quality and quantity of DNA was checked using gel electrophoresis and UV spectrophotometer.

c. Measurement of DNA Concentration

The concentration of DNA was measured spectrophotometrically. 1 µl of DNA sample was dissolved in 999 µl of distilled water and the absorbance was read at 260 nm. The concentration of DNA was recorded from the spectrophotometer.

The DNA Samples were diluted to make 50 ng μl^{-1} (after calculating the dilution factor) by adding T10 E1 buffer.

d. Purity of Isolated DNA

The purity of DNA was checked by running the samples on 0.8 per cent agarose gel.

e. PCR Amplification of DNA

The reaction mixture composition for the polymerase chain reaction was prepared by using the following reagents for each of the DNA sample.

10 x PCR buffer (Invitrogen Life technologies, USA): 2µl
2.5 mM dNTPs (American Biosciences, UK): 1 µl
Primer (Operon Technologies, USA): 1 µl
$MgCl_2$ (80 mM) (Invitrogen Life technologies, USA): 0.6 µl
Taq polymerase (Invitrogen Life technologies, USA): 0.2 µl
Template DNA (50 ng μl^{-1}): 3 µl
Milli- Q- Water: 12.2 µl

f. Primer survey and selection

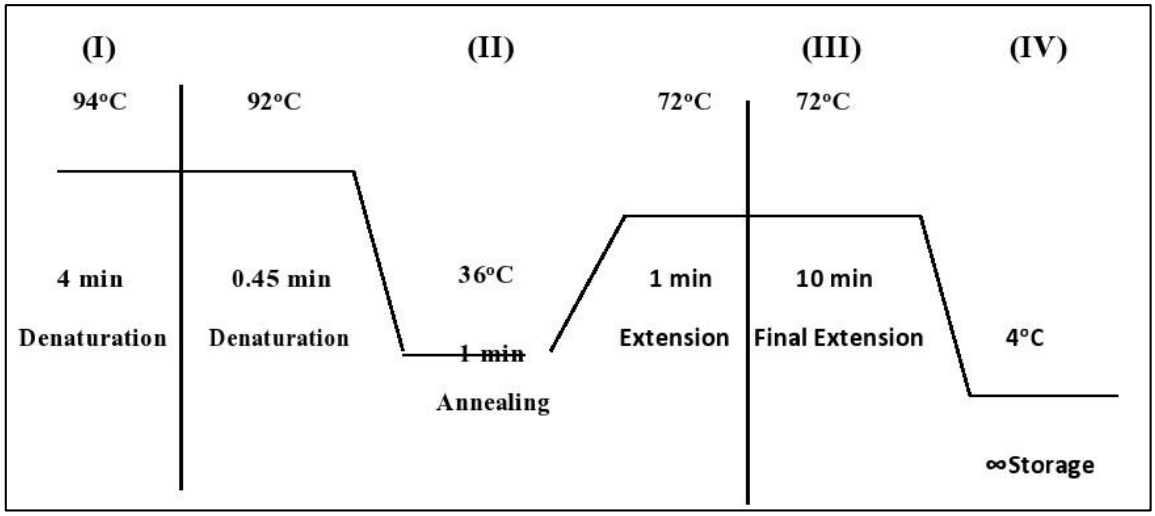
Preliminary primer screening was carried out with 60 primers (Ten- Base oligonucleotide) and 20 primers from OPA, OPC, OPD and OPH series (Operon Technologies, Inc., USA) were employed for molecular variation analysis. The primers that gave reproducible and scorable amplifications were used in the analysis of genetic variability of the isolates.

Table 2: List of primers used in RAPD study

S. No	Primers name	Sequence
1	OPA-02	TGCCGAGCTG
2	OPA-03	AGTCAGCCAC
3	OPA-05	AGGGGTCTTG
4	OPA-06	GGTCCCTGAC
5	OPA-07	GAAACGGGTG
6	OPA-09	GGGTAACGCC
7	OPA-10	GTGATCGCAG
8	OPA-17	GACCGCTTGT
9	OPA-18	AGGTGACCGT
10	OPC-18	TGAGTGGGTG
11	OPD-1	ACCGCGAAGG
12	OPD-5	TGAGCGGACA
13	OPD-7	TTGGCACGGG
14	OPD-11	AGCGCCATTG
15	OPD-15	CATCCGTGCT
16	OPD-16	AGGGCGTAAG
17	OPH-7	CTGCATCGTG
18	OPH-15	AATGGCGCAG
19	OPH-16	TCTCAGCTGG
20	OPH-18	GAATCGGCCA

Master mix was prepared by mixing all the components except template DNA in a separate eppendorf tube. Then 17 µl of master mix was added to another tube containing 3 µl of template DNA and a spin was given. The PCR tubes were capped and kept in PCR machine (MJ Research, USA) by the following programme.

The reaction mixture composition for the polymerase chain reaction was prepared by using the following reagents for each of the DNA sample.



g. Electrophoresis of PCR Products

The amplified DNA samples for electrophoresis were prepared by mixing loading buffer (6x). The bromophenol blue indicates the position of run and sucrose present in the loading buffer helps the DNA sample to settle down at the bottom of the well.

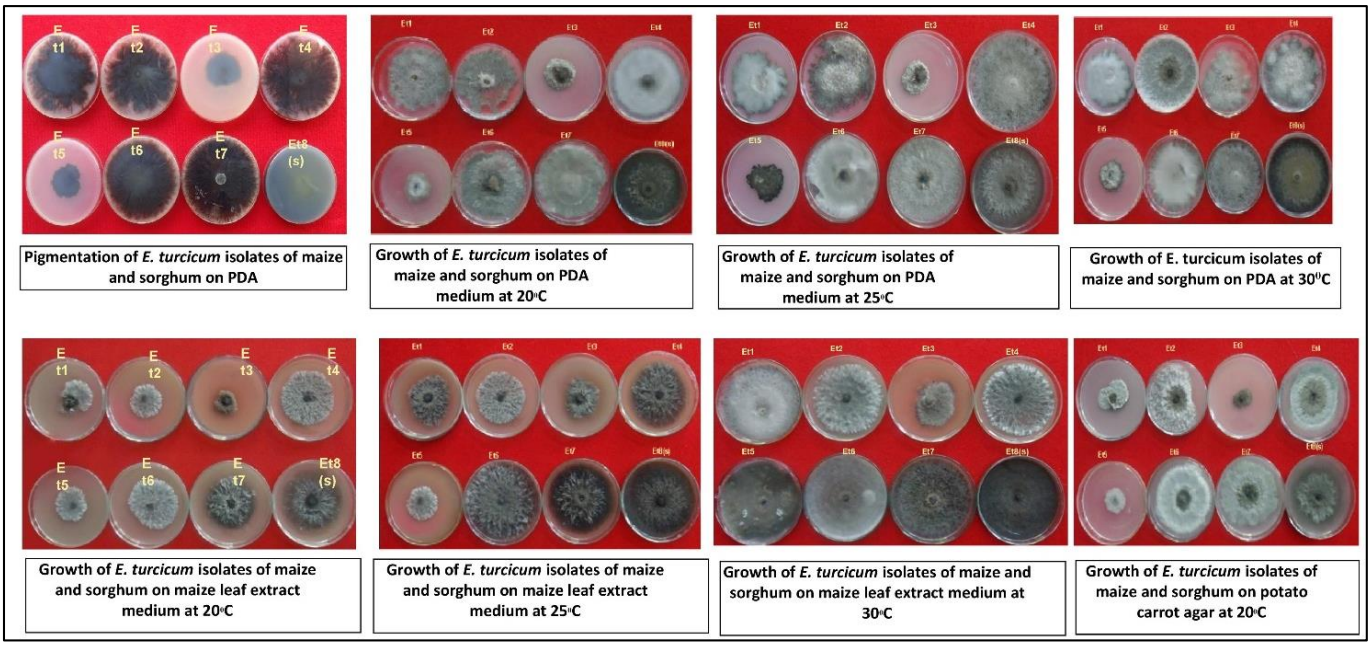
Agarose gel (1.5%) was prepared and placed in electrophoretic tank (Bio- Rad, USA) and submerged in 1 x TAE buffer. Samples were loaded in the wells using a micro pipette and electrophoressed at 90 V 30 minutes and the separation of DNA sample was checked on UV transilluminator and photographed using gel documentation system (Gene flash, Syngene Biosciences, USA).

The RAPD procedure was repeated twice for each of the primer to test the reproducibility of DNA bands. The data generated from each primer gel run was recorded in a matrix identifying the presence (1) or absence (0) of each RAPD band of a particular molecular weight. A similarity matrix was generated from the binary data using DICE similarity co-efficient in SIMQUAL programme of NTSYS-pe package (Rohlf, 1993) [18].

Results & Discussion

Cultural variability studies

Cultural variability of different Et isolates in respect of colony colour, pigmentation, colony character, sporulation is presented hereunder (Table 3 & 4; Fig 1, 2).



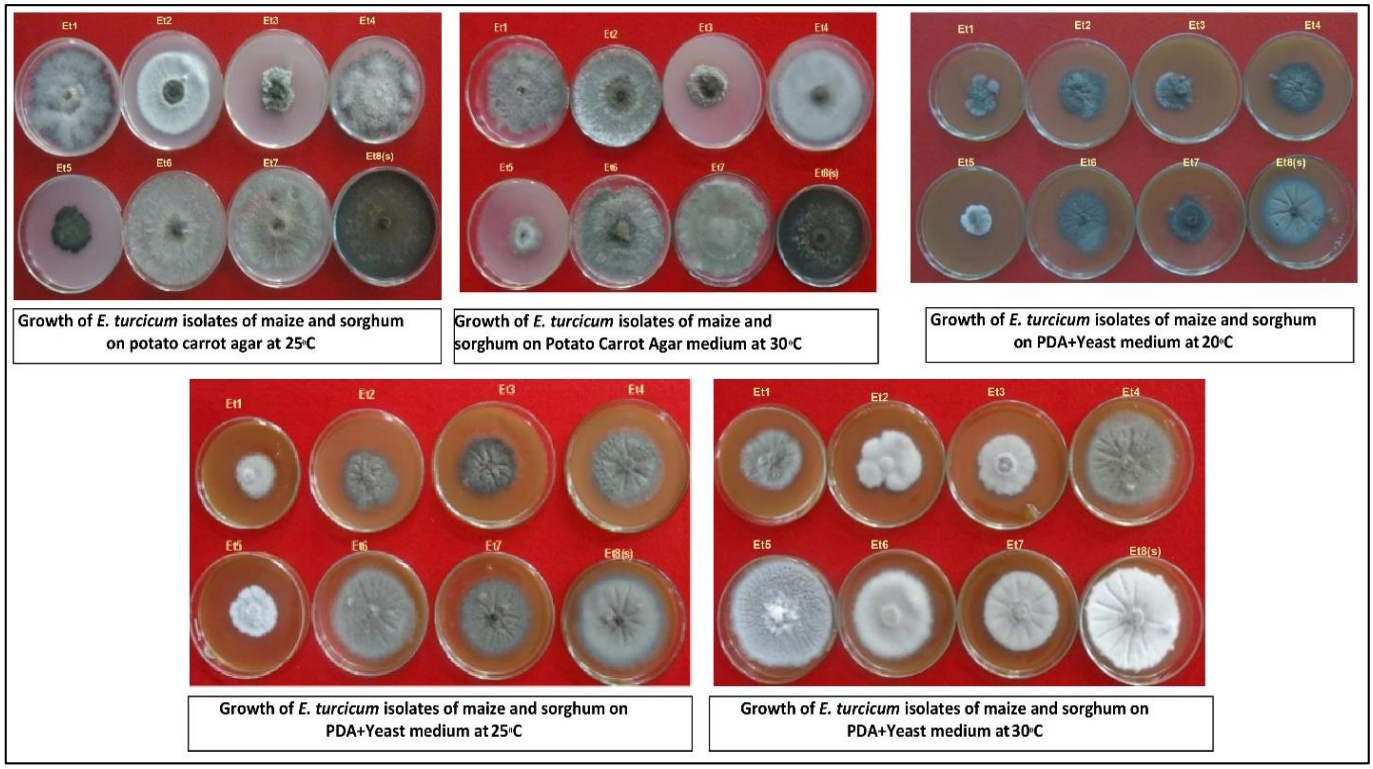


Fig 1: Cultural variability of *Exserohilum turcicum* on different growth media

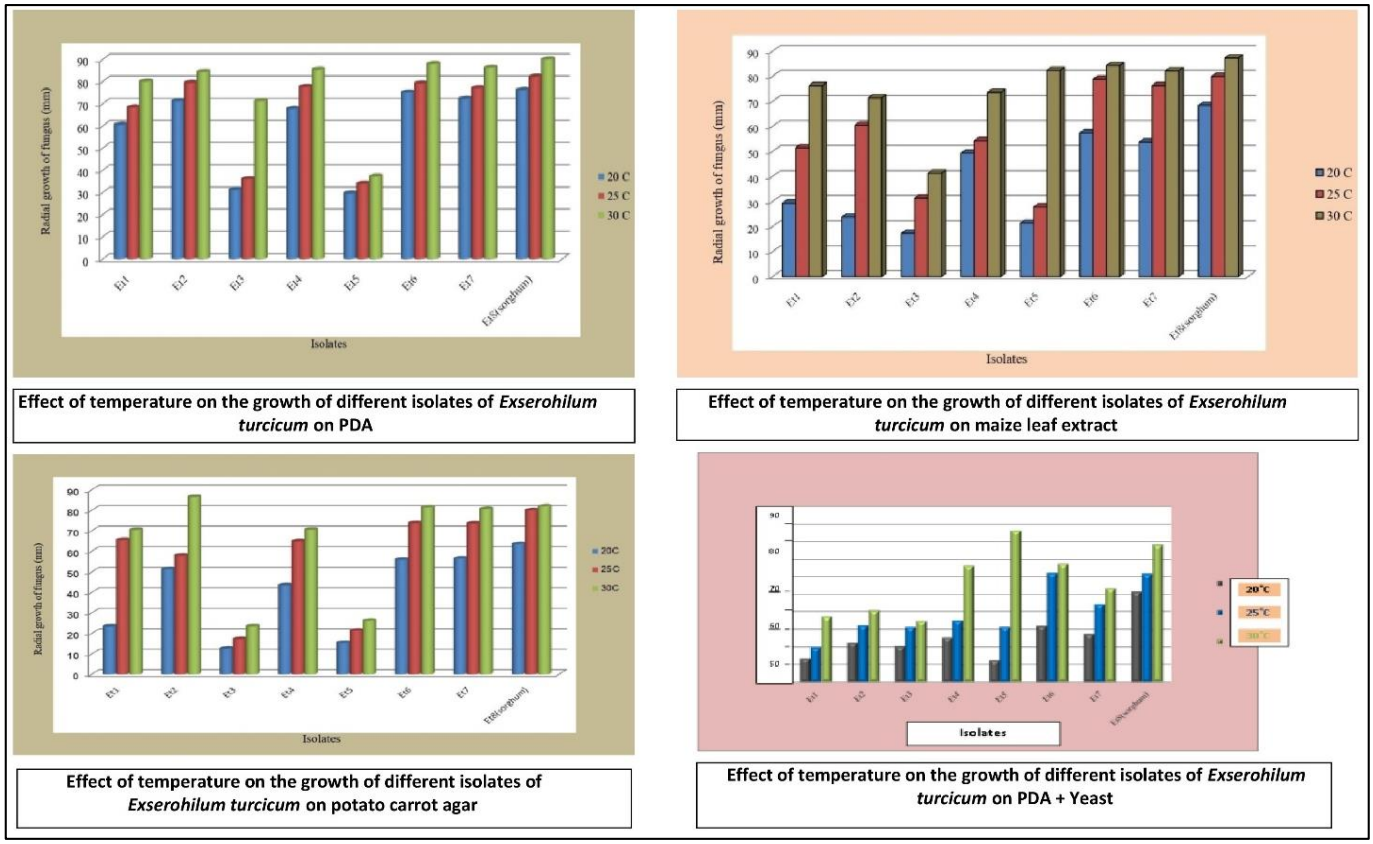


Fig 2: Effect of temperature on the growth of different isolates of *Exserohilum turcicum* on different growth media

Table 3: Cultural Characters of different isolates of *Exserohilum turcicum* collected from maize and sorghum

S. No	Isolates	Colony colour	Pigmentation	Colony character	Sporulation
1	Et1	Gray (2.5 Y 5/1)	Black (16 YR 2/1)	Moderate growth, appressed in centre, slightly fluffy on margin, black in centre, grayish colony, with irregular margin.	++++
2	Et2	Dark gray (2.5 Y 4/1)	Bluish black (2.5/1 10P)	Moderate growth, blackish in centre, dark gray colony with irregular margin	+++
3	Et3	Very dark grayish brown (2.5 Y 3/2)	Bluish black (2.5/1 10P)	Poor restricted growth, centre light black, very dark grayish brown colony with irregular margins.	+++
4	Et4	Gray(2.5 Y 5/1)	Black 16 YR 2/1	Moderate growth, black in centre, gray color regular margin.	++++
5	Et5	Very dark gray (2.5 Y 3/1)	Bluish black (2.5/1 10P)	Poor restricted cottony growth throughout very dark gray colony with irregular margin.	+
6	Et6	Very dark gray (2.5 Y 3/1)	Black (16 YR 2/1)	Profuse growth, slightly fluffy on margin, very darkgray color colony with circular margin	++++
7	Et7	Dark gray (2.5 Y 4/1)	Black (16 YR 2/1)	Profuse growth, appressed in centre, dark gray colony with regular margin.	++++
8	Et8(sorghum)	Black (2.5Y /1)	Greenish black (5G 2/1)	Profuse growth , dark black in centre, fluffy grayish black circular margin.	+++

The colour of the colony was determined with the help of Munsell's soil colour chart (Soil Survey Staff, 1951)
++++ - Excellent->20conidia per microscopic field Profuse growth - > 80 mm growth in petri plate
+++ - Good –15-20 conidia per microscopic field Moderate growth – 60-70mm growth in petri plate
++ - Fair- 10-15 conidia per microscopic field Poor growth - < 60 mm growth in petri plate

Table 4: Effect of different media and temperature on the growth of different isolates of *Exserohilum turcicum*

Colony diameter of the fungus (mm)													
S. No	Isolates	PDA			Maize leaf extract			Potato carrot agar			PDA + Yeast medium		
		20 ^{0}C	25 ^{0}C	30 ^{0}C	20 ^{0}C	25 ^{0}C	30 ^{0}C	20 ^{0}C	25 ^{0}C	30 ^{0}C	20 ^{0}C	25 ^{0}C	30 ^{0}C
1	Et1	60.53	68.33	80.00	29.33	51.43	76.20	23.33	65.43	70.36	13.30	19.73	37.23
2	Et2	71.20	79.40	84.33	23.80	60.46	71.36	51.33	57.80	86.56	22.20	32.20	40.73
3	Et3	31.20	36.00	71.23	17.23	31.33	41.23	12.46	17.23	23.40	20.30	31.26	34.60
4	Et4	67.67	77.56	85.36	49.26	54.23	73.50	43.56	64.83	70.50	25.23	35.06	66.06
5	Et5	29.63	34.00	37.20	21.36	27.76	82.33	15.36	21.36	26.16	12.36	31.30	86.16
6	Et6	75.06	79.13	88.00	57.36	78.83	84.26	55.80	73.70	81.43	31.90	62.00	67.16
7	Et7	72.23	77.00	86.16	53.66	76.23	82.16	56.33	73.50	80.63	27.23	44.23	53.20
8	Et8(sorghum)	76.16	82.16	90.00	68.30	79.90	87.20	63.46	80.00	81.90	51.30	61.56	78.23
	CD	2.174	2.06	1.96	1.87	1.79	1.68	1.94	2.30	2.26	1.96	2.04	2.10
	SE(d)	1.017	0.96	0.91	0.87	0.83	0.78	0.91	1.07	1.04	0.92	0.95	0.98
	SE(m)	0.719	0.68	1.45	0.62	0.59	0.55	0.64	0.76	0.73	0.65	0.67	0.64

Morphological variability studies

Conidial length

The data on the variability in the morphological characters of the eight isolates were presented in Table 5 (Fig. 3) and the differences in conidial length of all the isolates were significant. The isolates Et1 (Nadia) recorded the maximum (117.26 µm) conidial length and minimum (82.50 µm) conidial length was recorded by the isolate Et5 from Murshidabad district. The differences observed between Et3 (Bankura), Et4 (Birbhum) and Et7 (Purba Medinipur) isolates with a conidial length of 107.00 µm, 109.33 µm and108.33 µm respectively were non-significant. While the conidial length was 101.00 µm in case of Et2 isolate from Bardhaman and it was on par with the isolate Et6 from Purulia district. The sorghum isolate Et8 has recorded 106.16 µm conidial length and was on par with the maize isolates Et3 (Bankura) only.

Conidial width

Maximum conidial width of 28.83 µm was recorded in isolate Et1 from Nadia and the minimum (20.66 µm) conidial width was recorded by the isolate Et7 from Purba Medinipur district. The isolate Et5 from Murshidabad district has recorded the 21.16 µm conidial width, though the conidial length was less and it was on par with isolates Et3 (22.50 µm) and Et7 (20.66 µm) from Bankura and Purba Medinipur districts. However, sorghum isolate Et8 has recorded the conidial width of 23.00 µm and was on par with the isolate Et3 (Bankura) and Et6 (Purulia). The differences in conidial width of Et1 and Et2 were on par.

Conidial size

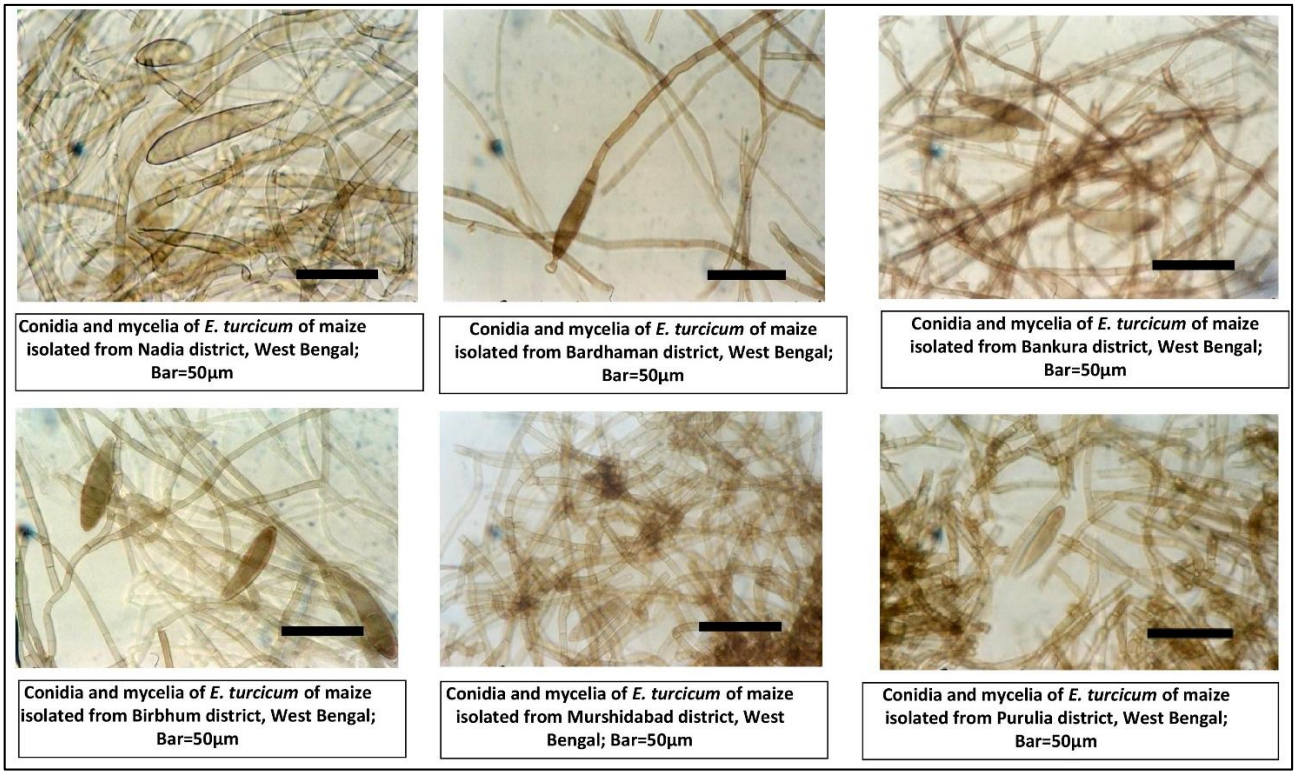
Differences were observed in conidial size (Length×Width) of all the eight *E. turcicum* isolates (Table 5). Maximum conidial size of 3352.13 µm^2 was recorded in Et1 (Nadia) isolate while minimum (1757.0 µm^2) conidial size was recorded by the isolate Et5 (Murshidabad). The conidial size of isolate Et2 from Bardhaman (2736 µm^2) was on par with isolate Et4 (2843.33 µm^2) from Birbhum district. Similarly the differences observed in conidial size between sorghum isolate Et8 (2446.33 µm^2) and two maize isolates *i.e* Et3 (Bankura) and Et6 (Purulia) 2432.66 µm^2 and 2384.33 µm^2 respectively were non significant.

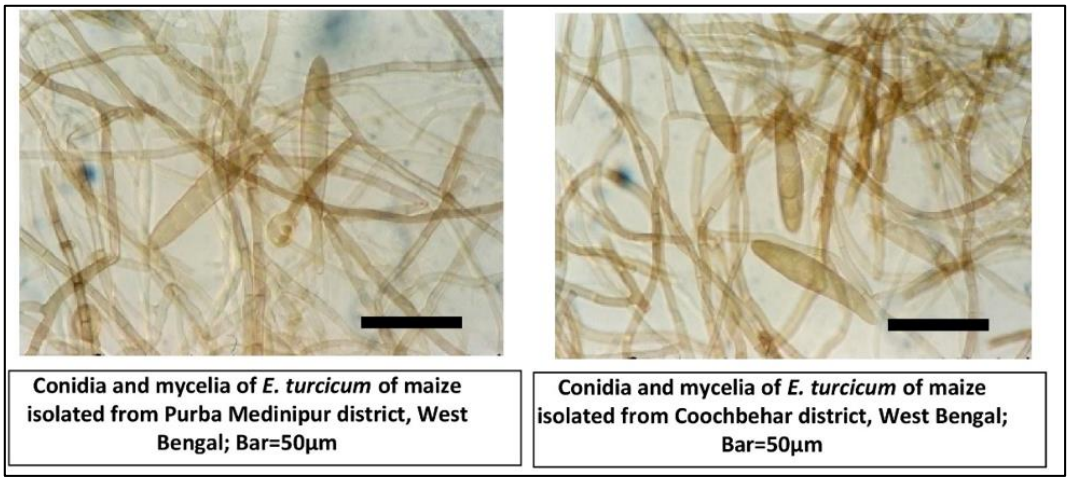
Number of septa

The number of septa per conidium were Murshidabad isolate which recorded lowest conidial size has recorded highest number of septa. The number of septa between isolates Et6 (5.66), Et7 (5.86) and Et8 (5.46) from Purulia, Purba Medinipur districts and sorghum were on par. Similarly the difference between isolates Et3 (Bankura) and Et4 (Birbhum) with 6.80 and 6.63 number septa respectively were on par with each other. Although Et1 (Nadia) recorded maximum conidial size but it recorded 7.40 septa only (Fig3).

Although some of the isolates produced length wise bigger conidia but their width was very small and some conidia were lengthwise smaller and widthwise they were wider. The

maximum conidial size was recorded by the isolate Et1 (Nadia) while the isolate Et5 from Murshidabad district recorded the minimum conidial size. The maximum number of septa was noted in the isolate Et5 from Murshidabad district, while the minimum number of septa was recorded by the sorghum isolate.





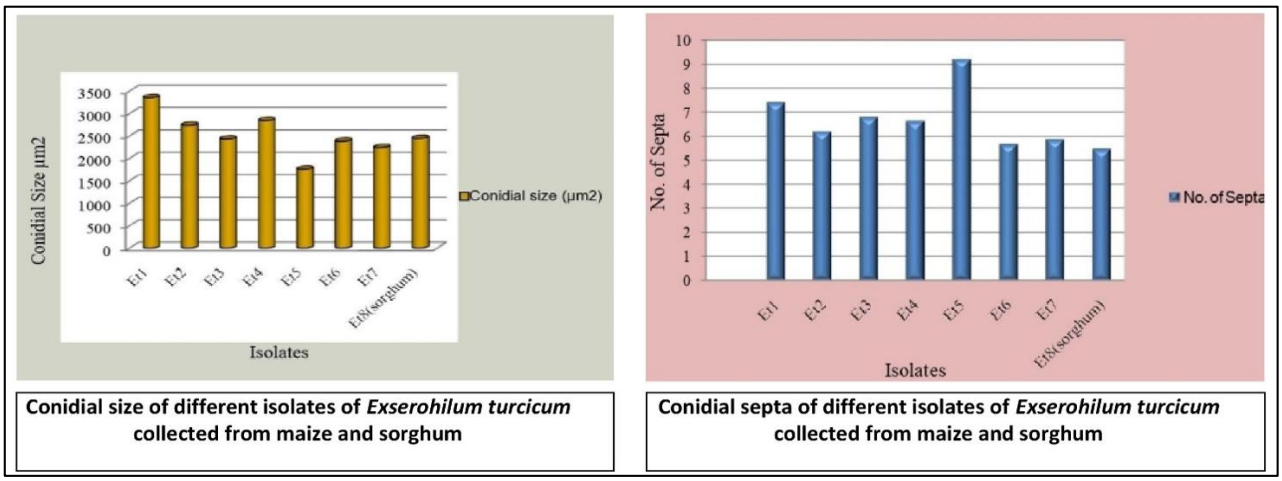


Fig 3: Morphological variability of *Exserohilum turcicum*

Morphological variation observed among different isolates of in the present studies are in confirmity with the work of Misra and Mishra, 1971 [12], Bergquist and Masias, 1974 [4], Daniel and Narong, 2006 [8], Varma *et al*., 2005 [20] and Bunker and Mathur, 2010 [5]. Despite these variations, the size and septa of conidia observed well with are in the standard description of conidial size (50-144× 18-33µm) and septa of 4-9 of *E. turcicum* (CMI, 1971) [6]. Bach and Kimati (1995) [3] made morphological comparisons among the isolates of *E. turcicum* (*Setosphaeria turcica*) obtained from maize, sorghum and Johnson grass (*Sorghum halepense*) and revealed that diameter of conidia of isolates from *S. halepense* were significantly larger than those from maize and sorghum.

Table 5: Morphological Characters of different isolates of *Exserohilum turcicum* collected from maize and sorghum

S. No	Isolates	Conidial length (µm)	Conidial width (µm)	Conidial size (µm2)	No. of Septa
1	Et1	117.26	28.83	3352.13	7.40
2	Et2	101.00	27.00	2736.00	6.20
3	Et3	107.00	22.50	2432.66	6.80
4	Et4	109.33	26.00	2843.33	6.63
5	Et5	82.50	21.16	1757.00	9.20
6	Et6	99.41	24.00	2384.33	5.66
7	Et7	108.33	20.66	2239.33	5.86
8	Et8 (sorghum)	106.16	23.00	2446.33	5.46
	CD	2.11	1.48	176.77	0.58
	SE(d)	0.98	0.69	82.67	0.27
	SE(m)	0.69	0.48	58.46	0.19

Genetic variability studies

Polymorphism was observed among the seven *Exserohilum turcicum* isolates of maize and sorghum. RAPD analysis of seven *Exserohilum turcicum* isolates of maize and one *Exserohilum turcicum* isolate of sorghum was done with ten base pair oligonucleotide operon polymers. Out of 60 polymers used for amplification, 20 were able to amplify the DNA of all the seven isolates of maize and one sorghum isolate. Polymorphism of the *Exserohilum turcicum* isolates of maize and sorghum with different primers were shown in Fig 4. The number of amplified products were highest for the primer OPH7, followed by OPD11, OPD13 and OPA9. Least number of products was generated by OPA6 followed by OPC16 and OPH15. Information on banding pattern with all primers were used to determine the similarity co-efficient and genetic distance between isolates and further to construct dendrogram (Fig 5).

Coefficient values of each isolate derived from RAPD study were presented in the Table 6. Among the 7 maize isolates of test pathogen highest similarity co-efficient 0.85 was shown between isolates of Bankura and Birbhum followed by 0.70 between isolates of Nadia and Bankura, and between isolates of Nadia and Birbhum. Lowest similarity coefficient 0.51 was seen between Murshidabad and Nadia isolates, followed by 0.53 between Murshidabad and Bardhaman isolates. However, sorghum isolates have shown highest similarity coefficient with Birbhum and lowest similarity coefficient with Murshidabad.

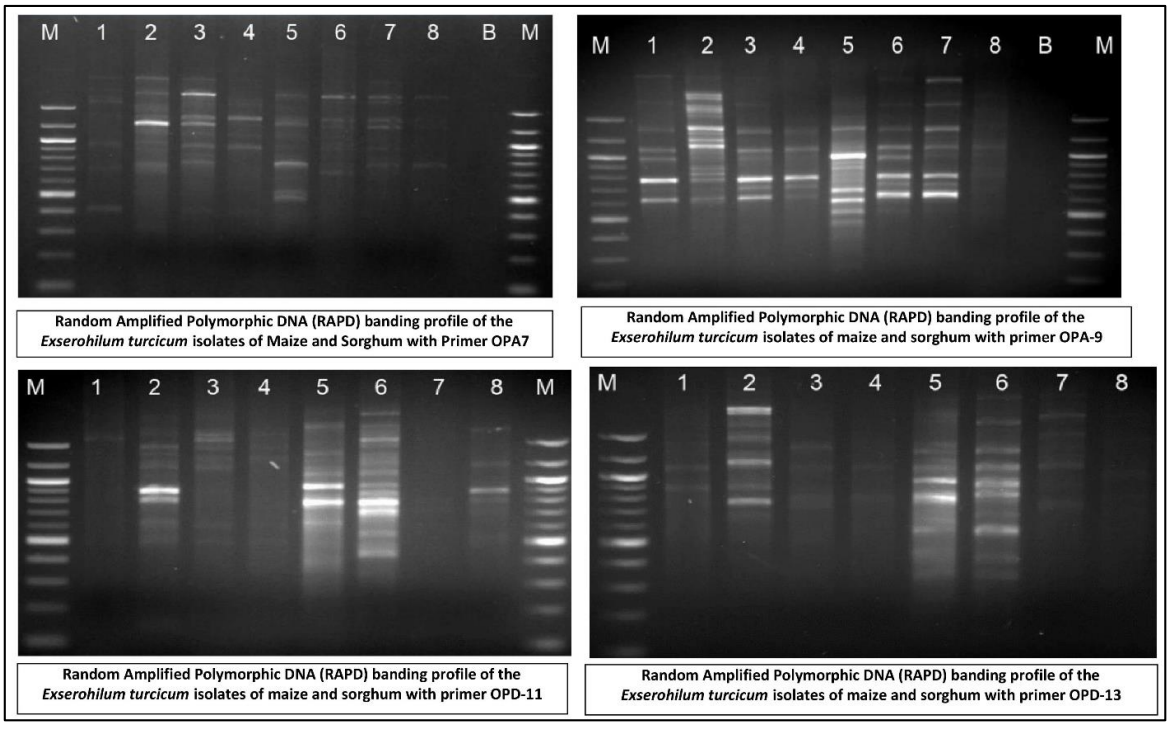


Fig 4: Genetic variability of Exserohilum turcicum based on RAPD banding profile, M: Marker (100 bp DNA ladder); 1 to 8: Seven Exserohilum turcicum isolates from maize and one from sorghum; 1: Nadia; 2: Bardhaman; 3: Bankura; 4: Birbhum; 5: Murshidabad; 6: Purulia; 7: Purba Medinipur; 8: Sorghum.

Data differentiated the test isolates into 2 major clusters A and B (Fig 5). Cluster A was divided into sub-clusters A1 and A2. A1 was further classified in to A3 and A4; A3 was differentiated into A5 and A6. A5 was further divided into A7 and A8. cluster A2 includes Purulia and Purba Medinipur isolates, A4 includes Bardhaman isolate, A6 includes sorghum isolate, A7 includes Nadia isolate, A8 includes Bankura and Birbhum isolates; cluster B includes Murshidabad isolate. In the present study, the RAPD analysis revealed genetic diversity among isolates of *E. turcicum* which was notably higher according to banding pattern observed. Out of 60 primers initially evaluated for amplification, 20 primers generating reproducible banding patterns were selected. Among these primers, three primers OPH7, OPD11 and OPD-13 have generated more polymorphic bands.

The Random Amplified Polymorphic DNA is a PCR based technique which reveals polymorphism with in completely unknown samples without the need of probe hybridization or DNA sequencing. RAPDs have also been used in race, pathotype and population studies (Crowhurst *et al*. 1991 and Manualis *et al*. 1994) [7, 11]. This feature combined with their easy identification makes it a valid type of marker potentiality useful in many areas of genetic research such as gene mapping and strain differentiation of *Trichoderma* spp (Zimad *et al*., 1994) [22]. In present study, detection of genetic variability of *E. turcicum* isolates was done by PCR using 60 random primers. The results revealed that the isolates were polymorphic and confirmed the genetic variability. Among the isolates maximum genetic variation was high with minimum similarity coefficient of 0.50 between Bardhaman and Purba Medinipur district.

These findings have clearly shown that RAPD can genetically differentiate the isolates at inter specific level. No clustering was observed according to location. The cluster formed was not even coincided with virulence except for Murshidabad isolate for the morphological characters. The Murshidabad isolate shows entirely different morphological characters than other isolates. The isolates that were identical for virulence were dissimilar with RAPD markers. In each group or sub group isolates from different locations were present indicating high genetic diversity. The most virulent Birbhum and Purulia isolates were grouped in different sub groups.

Table 6: Similarity coefficient values of *Exserohilum turcicum* isolates of maize and sorghum based on RAPD analysis

	Et1	Et2	Et3	Et4	Et5	Et6	Et7	Et8
Et1	1.00							
Et2	0.58	1.00						
Et3	0.70	0.69	1.00					
Et4	0.70	0.68	0.85	1.00				
Et5	0.51	0.53	0.58	0.59	1.00			
Et6	0.57	0.50	0.57	0.57	0.58	1.00		
Et7	0.60	0.60	0.65	0.60	0.54	0.62	1.00	
Et8	0.62	0.64	0.66	0.69	0.51	0.56	0.63	1.00

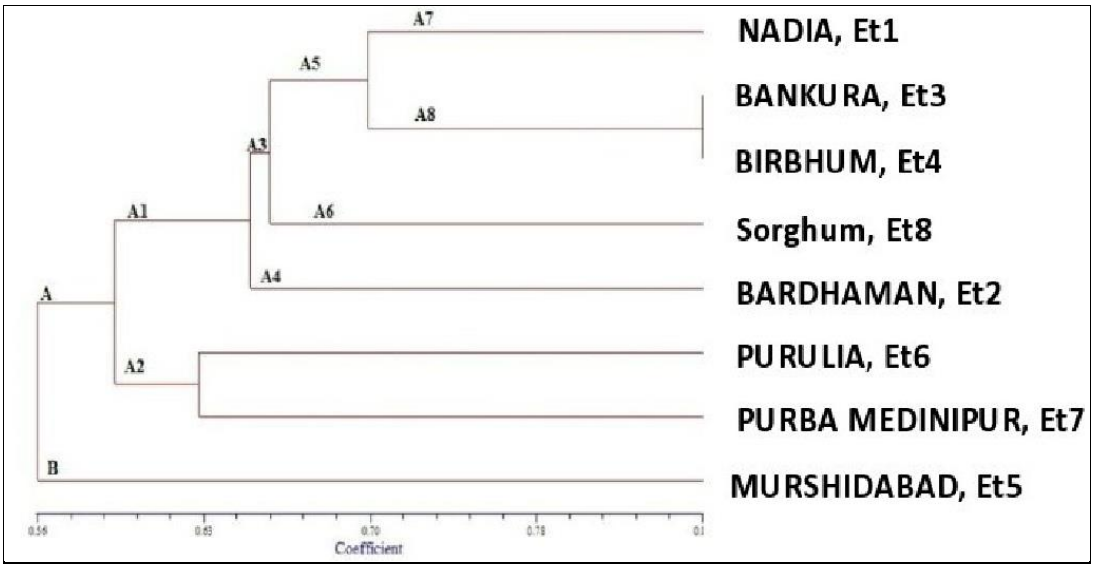


Fig 5: Grouping of *Exserohilum turcicum* isolates of maize and sorghum based on RAPD banding pattern

Conclusion:

The eight (8) *Exserohilum* isolates (Et1 – Et8) collected from different districts of West Bengal *viz.*, Nadia, Bardhaman, Bankura, Birbhum, Murshidabad, Purulia, Purba Medinipur and Coochbehar districts during *Kharif* 2017-18 to assess the disease severity and to study variability of the pathogen. The eight single spore-cultures of *E. turcicum* isolates (Et) exhibited cultural variability in respect of colony colour, colony growth, sporulation and pigmentation on best suited four media such as PDA, MLEA, PCA and PDA+Yeast at different temperature ranges. Colony colour varied from grey to black in colour depending upon the isolates and growth medium. The isolate Et4 (Birbhum), Et6 (Purulia), Et7 (Purba Medinipur) and Et8 (sorghum, Coochbehar) were under the group of profuse to moderate growth was exhibited in the isolates Et1 (Nadia) and Et2 (Bardhaman) while poor restricted growth was observed in the Et3 (Bankura) and Et5 (Murshidabad) isolates. The isolates Et1 (Nadia), Et4 (Birbhum), Et6 (Purulia), Et7 (Purba Medinipur) produced excellent sporulation while the isolates Et2 (Bardhaman), Et3 (Bankura) exhibited good sporulation and showed the similarity with sorghum isolate Et8. The isolates Et1 recorded the maximum (117.26 µm) conidial length and minimum (82.50 µm) conidial length was recorded by the isolate Et5. The isolates that were identical for virulence were dissimilar with RAPD markers. The most virulent Birbhum and Purulia isolates were grouped in different sub groups.

Acknowledgement
First author of this manuscript is very much thankful to Prof. (Dr.) Parthasarathi Nath, Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, West Bengal and AICRP maize, BCKV for providing all experimental facilities, critical suggestions, and preparation of manuscript. This is a part of Ph.D. thesis of first author under the guidance of Prof. (Dr.) Parthasarathi Nath.

References

1. Anonymous. Project Director Review 2013-14: All India Co-ordinated Maize Research Project, Directorate of Maize Research, New Delhi, 2010, 2.
2. Anonymous. Project Director Review 2015-16: All India Co-ordinated Maize Research Project, Directorate of Maize Research, New Delhi, 2016, 8.
3. Bach EE, Kimati H. Morphological and pathogenic comparisons of *Exserohilum turcicum* isolated from maize, sorghum and jhonsons grass. Summa Phytopathologica. 1995;21(2):134-139.
4. Bergquist RR, Masias OR. Physiologic specialization in *Trichometasphaeria turcica* and *T. turcica* f. sp. *sorghi* in Hawaii. Phytopathology. 1974;64(5):645-649.
5. Bunker RN, Kusum M. Pathogenic and morphological variability of *Exserohilum turcicum* isolates causing leaf blight in sorghum (*Sorghum bicolor*). Indian Journal of Agricultural Sciences. 2010;80(10):888-892.
6. CMI. Descriptions of Pathogenic Fungi and Bacteria. No. 304. *Trichometaspaeria turcica*. 1971, CAB, UK.
7. Crowhurst RN, Hawthorn BT, Rikkerink EH, Templeton MD. Differentiation of *Fusarium solani* f. sp. *cucurbitae* race 1 and 2 by random amplification polymorphic DNA. Cuurent Genetics. 1991;2:931-936.
8. Daniel A, Narong S. Morphological, Cultural and Pathogenicity variation of *Exserohilum turcicum* (Pass) Leonard and Suggs Isolates in Maize (*Zea mays* L.). Kasetsart Journal (Nat. Sci.). 2006;40(2):341-352.
9. Haralpur SI, Kulakarni MS. Conidial germination of *Exserohilum turcicum* (Pass.) Leonard and Suggs. causing turcicum leaf blight of maize. Journal of Plant Disease Science. 2009;4(2):231-231.
10. Ho WC, Ko WH. A simple method for obtaining single spore isolation of fungi. Botanical Bulletin of Academia Sionica.1997;38:41-44.
11. Manualis S, Kogan N, Reuven M, Ben, Yephet Y. Use of the RAPD technique for identification of *Fusarium oxysporum* f sp *dianthi* from Carnation. Phytopathology. 1994;84(1):98-101.
12. Misra AP, Mishra P. Variations in four different isolates of *Helminthosporiurn turcicum* from *Sorghum vulgare*. Indian Phytopathology. 1971;24**:**514-521.
13. Muiru WM, Mutitu EW, Kimenju JW. Distribution of turcicum leaf blight of maize in Kenya and cultural variability of its causal agent, *Exserohilum turcicum*. Journal of Tropical Microbiology and Biotechnology. 2008;4(1):32-39.
14. Payak MM, Sharma RC and Lilaramani J. How to control maize diseases. Indian Farming. 1973;23:20-23.
15. Payak MM and Sharma RC. Maize diseases and approaches to their management. Trop. pest management. 1985;31(4):302-310.
16. Perkins JM, Pederson WL. Disease treatment and yield loss associated with northern leaf blight of corn. Pl. Dis. 1987;71(10):940-943.
17. Raymundo AD, Hooker AC. Measuring relationship between northern leaf blight of maize and yield losses. Pl. Dis. Bull. 1981;65:325-327.
18. Rohlf FJ. NTSYS Pc. Numerical taxonomy and multivariate analysis system. Exter Publishing, Setauket, New York. 1993;159:239-241.
19. Smith DR. Global disease assessment of corn. In: Proc. fifty-fourth Annual Corn and Sorghum Res. Conf., December 9-10, Chicago, 1999, 54.
20. Varma PK, Hegde YR, Kulkarni S, Kalappanavar IK. Variability in *Helminthosporium sativum* with respect to morphology and symptomatology. Annals of Biology. 2005;21(2):209-212.
21. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic acids Research. 1990;18(22):6531-6535.
22. Zimad G, Lea V, Elad Y, Chet I, Manualis S. Use of RAPD procedure for the identification of *Trichoderma strains*. Mycological Research. 1994;98(5):531-539.