Original Research Article

Isolation and Characterization of Pathogen causing Northern Corn Leaf Blight of Maize in Ri-Bhoi, Meghalaya, India.

ABSTRACT:

The present study was carried out to identify and characterize the pathogen causing northern corn leaf blight of maize based on the cultural, morphological and molecular characteristics. Diseased leaf samples from maize fields exhibiting typical symptoms of northern corn leaf blight were collected from the research fields ofCPGS-AS (Umiam), College of Agriculture (Kyrdemkulai) and ICAR-RC for NEH region (Umiam) located in Ri-Bhoi district, Meghalaya. Through cultural, morphological, molecular characterisation the fungal isolate was identified as *Exserohilum*sp. Phylogenetic analysis of ITS rDNA gene sequence indicates as *Exserohilumturcicum*is associated with the disease northern corn leaf blight of maize. The findings underscore the importance of pathogen *Exserohilumturcicum*causing northern corn leaf blight impact on the maize crop, highlighting potential threat toits production.

Keywords: Characterisation, *Exserohilumturcicum*,Maize, Northern Corn Leaf Blight,

1.INTRODUCTION:

Maize (*Zea mays* L.), the world's third most significant staple crop after paddy and wheat. As a native of Mexico and Central America, maize (*Z. mays* L.), with chromosome number 2n=20, belongs to the poaceae family and is one among the most adaptable emerging crops, demonstrating wider adaptability under varied agro-climatic conditions (Dowswell*et al*., 1996). Because it has the highest genetic yield potential of all cereals, maize is known worldwide as the "queen of cereals," and it is grown on nearly 197 m ha, with a production of 1,220,542 MT in approximately 170 countries with wider diversity of soil, climate, biodiversity, and management practices. This crop accounts for 39 % of the world's grain production.

India is the fourth-largest and sixthmost productive nation among those that cultivate maize, accounting for 3 % of global production and 4 % of the world's total acreage. In India, maize is grown all year round in a variety of climates, from extremely semi-arid to subhumid and humid areas.After rice and wheat, it is the third-most significant cereal crop in India,

making up around 10 % of the nation's total food grain production (IIMR, 2023). In India, an area of 9.3 million hectares was grown with maize, an anticipated production of 31.56 million tonnes and productivity of 3.19 t/ha in 2022-2023 (Anonymous, 2023a).

Queen of cereals, maize is the second most important food crop in Meghalaya, after rice. It covers around 18,000 hectares (8 % of the total area), produces 40,764 tonnes, and yields an average of 2150 kg/ha. The state's average productivity is lesser than the nation's (Babu *et al*., 2019). Maize is grown in all the 12 districts of Meghalaya. Ri-Bhoi is the leading district in maize growing area (3142 ha) and production Ri-Bhoi (10,152 Tonnes) (Anonymous, 2023b).

Maize is affected by several diseases caused by fungi, bacteria, virus, nematodes which leads to an economic loss of 13.2 % (Kumar *etal*., 2014). Northern corn leaf blight (NCLB) also called as turcicum leaf blight (TLB) caused by *Exserohilumturcicum* (Pass.) Leonard and Suggs(Harlapur*et al*., 2000).

NCLB show the symptoms like long cigar-shape grey-green to tan coloured lesions on lower leaves. Tan lesions are slender and oblong tapering at the ends ranging in size from 1-6 inches. Lesions run parallel to leaf margins and they coalesce and cover entire leaf. Spores are produced on the underside of leaf. Below the lesions, fungus giving the dusty black/green fuzz appearance. Leaves become greyish-green and brittle, resembling leaves killed by frost. Subsequently, lesion induced spores rapidly disperse the disease (Reddy *et al*., 2014). The pathogen can overwinter in soil and plant residues, allowing it to survive unfavourable conditions. During favourable conditions, *E. turcicum* is dispersed by wind and rain splash (Hooda *et al*., 2017).

2.MATERIALS AND METHODS:

2.1 Collection and identification of northern corn leaf blight of maize

Maize leaves showing typical leaf blight symptoms were collected from maize fields of CPGS-AS (Umiam), College of Agriculture (Kyrdemkulai) and ICAR-RC for NEH region (Umiam). Each diseased sample was collected in separately in polythene bags with labelling and stored at 4 ^{0}C in refrigerator until further use. The collected diseased samples were examined under compound microscope to confirm the pathogens before isolation and conidia were observed under 10X and 40X magnification under compound microscope (Olympus CH2O i).

2.2 Isolation and purification of putative pathogen causing northern corn leaf blight of maize

2.2.1 Spore germination method

For the isolation of fungus, spore germination method of Turgay *et al*. (2020) was followed with a slight modification firstly the leaf samples were washed thoroughly under running tap water to remove dust and dirt particles. The small segments of diseased tissue along with some healthy leaf portions (5 x 5 mm^2) were cut with a sterilized razor blade at the margins of the diseased spots on the leaves and surface sterilized in 4 % sodium hypochlorite (NaOCl) solution for 30 seconds. The leaf segments were then rinsed thrice in distilled sterilized water to remove the last trace of sodium hypochlorite solution, blotted dry in filter paper to remove excess water and placed on the Petri plate with sterile blotting paper and then added a few drops of sterile distilled water to the blotting paper and the plates are incubated at 25 ± 1 ^{0}C for 3-4 days and then observed under microscope (10X and 40X magnification) to observe the spores of the putative pathogen.

Once the spores were produced then the leaf segment were transferred to the acidified potato dextrose agar medium (PDA) in sterilized Petri plates. Two to three pieces of sterilized specimen were placed in each Petri plates and incubated in BOD for 7 days at 25 ± 1 ^{0}C. Developing fungal colonies were sub cultured to obtain pure culture of the isolates and the obtained fungal isolates were sub cultured periodically to avoid contamination and for multiplication.

2.2.2 Hyphal tip isolation method

Hyphal tip isolation method of Meghana *et al*. (2023) was used for the isolation of the pathogen with a little modification. Infected leaf samples were cut into fragments of 3x3 mm^2 size with a combination of half portion of infected area and half portion of healthy area with the help of sterile razor blade. The fragments were disinfected with 4 % NaOCl solution followed by rinsing in sterile distilled water for 3 times and the leaf bits were dried on a sterile blotting paper for removing of excess water. Then the 2-3 leaf bits were transferred to sterilized Petri plates containing potato dextrose agar (PDA) in aseptic condition under laminar hood. The Petri plates were incubated at room temperature (25 ± 1 ^{0}C) for 7-10 days for fungal growth. The pure culture was obtained by subculturing the leading edge of hyphal tip of the fungal colony that grew from the leaf tissue sample onto PDA.

To obtain single conidial cultures, the pure culture grew for 7-10 days at 25 ± 1 ^{0}C until it sporulated.The pure cultures thus, obtained were maintained by repeated sub-culturing at an interval of 30 days for further studies. The stock culture in PDA slants were stored at 4 ^{0}C in refrigerator.

2.4 Identification of putative pathogen causing northern corn leaf blight of maize

The pathogens were identified on the basis of colony morphology characters *viz*., colour, shape, margins, growth, pigmentation *etc*. and morphological and microscopic characters of its mycelium, conidiophore and conidia produced such as number of cells, septation in culture were observed with the help of phase contrast microscope (LEICA) at 10X and 40X magnifications.

2.5 Pathogenicity test of northern corn leaf blight of maize

The pathogenicity test for putative pathogen of NCLB was carried out to prove Koch's postulates under controlled conditions at CPGS-AS, Umiam by following two methods to prove the pathogenicity.

2.5.1 Spray inoculation method

Spray inoculation method was done as described by Onwunali and Mabagala (2022) with slight modifications. Healthy maize seeds of variety Megha maize 2 were sown in the plastic pots containing sterilised soil and left for 30 days for vegetative growth. The leaves were surface sterilized with 70 % ethanol and then washed thoroughly with sterilized distilled water using hand atomizer. The spore suspension which was prepared as above was sprayed on both surfaces of those leaves with the help of hand atomizer. Control pot was maintained in which only sterilized distilled water was sprayed in order to compare with inoculated plants. After inoculation, the plants were covered with perforated polypropylene bag to maintain the moisture content and also not to spread of the inoculum for 48 hrs. Then the plants were regularly observed after 3 days for the appearance of leaf spot symptoms.

2.5.2 Detached leaf assay

Detached leaf assay method to prove pathogenicity of NCLB was followed as described by Muiru*et al*. (2008) with slight changes. Healthy leaves were detached from healthy 30 days and 60 days old maize plants. The leaves were surface sterilized with 4 % sodium hypochlorite (NaOCl) solution for one minute and then thoroughly washed with sterilized distilled water for two times. The leaves were transferred into Petri plates containing moistened sterilized blotting paper to maintain the humid conditions. Then the leaves were sprayed with the spore suspension of concentration $1x10^5$ spore/ml with the help of hand automizer. The inoculated plates were then incubated in the BOD incubator for 25 ± 1 ^{0}C. Control plate was also maintained for comparison where only sterilized distilled water was sprayed on the leaves. The leaves were observed daily for the appearance of leaf spot symptoms.

2.6 Molecular characterization and Phylogenetic analysis of pathogen causing northern corn leaf blight of maize

The fungal genomic DNA was extracted by employing the method described by Banerjee and Nath (2023) using HiPurA™ Fungal DNA Purification Kit. The obtained fungal genomic DNA was amplified using universal primers ITS 1F (5' TCCGTAGGTGAACCTGCGG 3') and ITS 4R (5' TCCTCCGCTTATTGATATGC 3')(Zahara *et al*., 2022). PCRamplification was carried out in 25 µl volume of PCR mixture, which contains 10X PCR buffer (3 µl), 10 mM dNTPs (2 µl), 1.5 mM $MgCl_2$ (1 µl), 10 mM (2.5 µl), Taq polymerase (0.2 units), nuclease free water (9.8 ml), 25 ng template DNA (4 µl). Amplification of the gDNA was performed in thermo cycler using PCR conditions, 94 ^{0}C for 2 min (initial denaturation), 94 ^{0}C for 1 min (denaturation), 54 ^{0}C for 1 min (annealing) and 72 ^{0}C for 1 min (extension). The amplification procedure was continued for 35 cycles and it terminated with a final step at 72 ^{0}C for 15 min. The PCR amplified product was separated on 1.5 % agarose gel in 1X TBE buffer (pH 8.0) in a gel electrophoresis unit and electrophoresed at 80V for 45 min.The PCR product of the sample was transferred into 1.5 ml sterilized Eppendorf tubes and sent for sequencing to GenOmbio Technologies India Pvt. Ltd., Pune, Maharashtra, India forITS rDNA sequencing.

The sequence data obtained from GenOmbio Technologies India Pvt. Ltd., Pune, Maharashtra, India was then analyzed with Basic Local Alignment Search Tool (BLASTn) search in online portal of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) for identity and homology search, the obtained sequence was then identified based on the high similarity index and the phylogenetic tree was constructed using MEGA 11 database software for evolutionary analysis by Maximum Likelihood method analysis at the nodes indicating the bootstrap values from 1,000 replications.

3 RESULTS:

3.1 Collection and identification of northern corn leaf blight of maize

The foliar fungal disease northern corn leaf blight of maize one of the most potential diseases of maize which is very common in the humid regions during the periods of heavy rainfall. Maize plants showing the symptoms of NCLB were identified and collected from the maize research fields of CPGS-AS (Umiam), ICAR-RC for NEH region (Umiam), College of Agriculture (Kyrdemkulai) in Ri-bhoi district, Meghalaya (Fig.1). Under the field conditions, it was found that the symptoms were first observed on the lower leaves characterized by minute chlorotic flecks and they are moving to the upper leaves during the course of time and spread all the leaves of maize plant. Mature symptoms of NCLB are characterized by grey-green coloured elliptical or cigar-shaped lesions that are 1-7" in length. As the disease

progresses, the lesions start to mature and become tan coloured with distinct dark zones that are related with fungal sporulation. In the advanced stages of the disease, multiple lesions can coalesce forming large irregular areas of dead tissue on the leaves. In severe

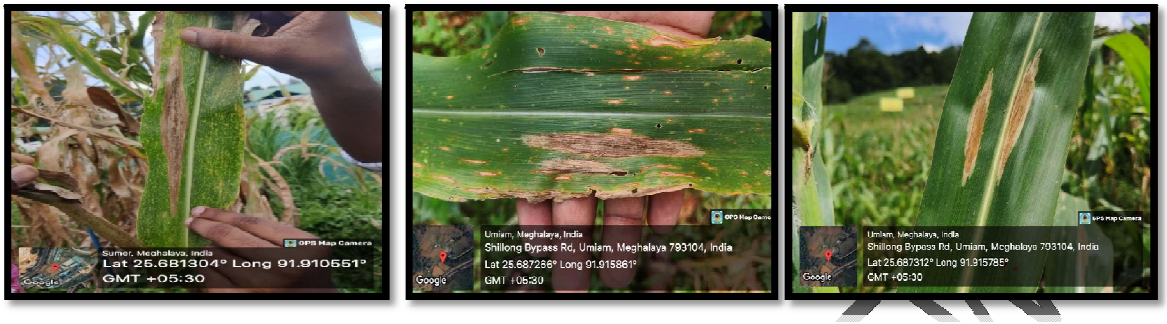


Fig. 1: Collection of northern corn leaf blight symptomed leaves from Ri-Bhoi district, Meghalaya

infection, almost all of the leaves may be infected and can be entirely blighted, resembling frost or drought injury. The pathogen was identified by observing the glass slide mount of diseased sample under 10X and 40X magnifications (Fig.2).

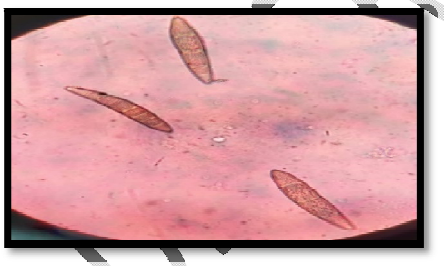


Fig. 2: Elongated- spindle shaped conidia from leaf samples of northern corn leaf blight of maize under 40X magnification

3.2 Isolation and characterization of pathogen causing northern corn leaf blight of maize

3.2.1 Isolation and purification of putative pathogen causing northern corn leaf blight of maize

The pathogen which causing NCLB was isolated by following two methods *viz*., i) spore germination method and ii) hyphal tip inoculation method on PDA medium. The isolated pathogen was purified by using hyphal tissue method and maintained by transferring into PDA slants and repeated sub culturing at an interval of 30 days. The PDA slants of the stock culture was maintained by storing at a temperature of 4 ^{0}C in refrigerator for the further use (Fig.3).

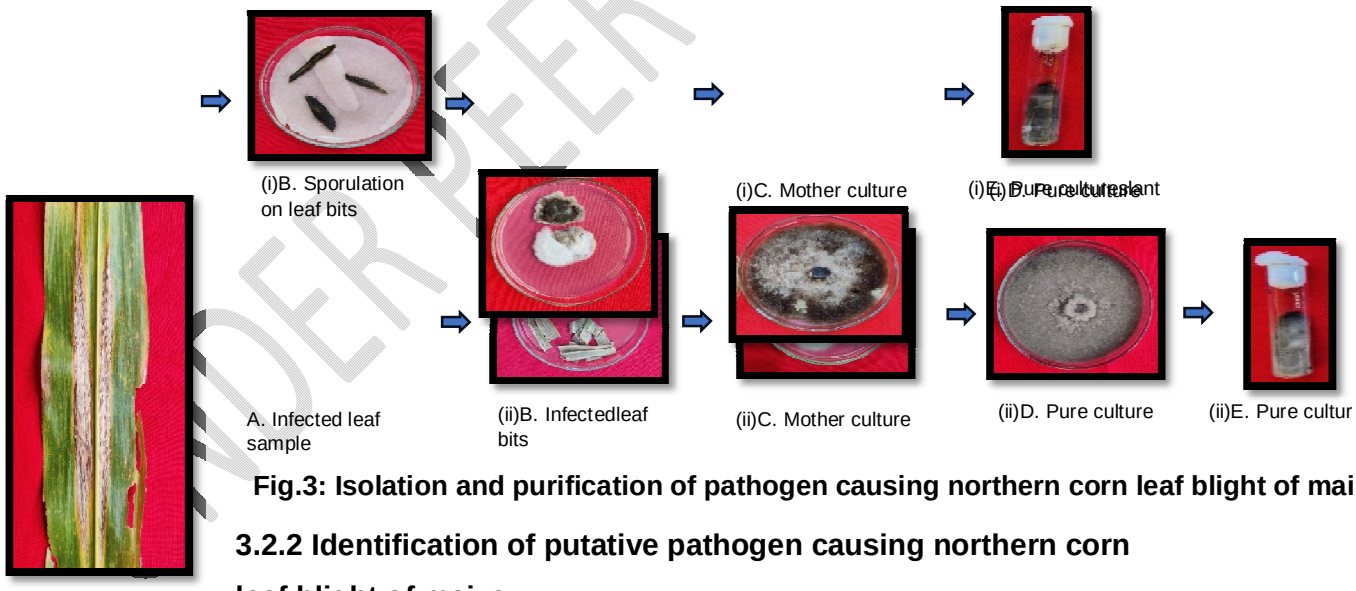


Fig.3: Isolation and purification of pathogen causing northern corn leaf blight of mai

3.2.2 Identification of putative pathogen causing northern corn leaf blight of maize

3.2.2.1 Cultural characteristics

Growth of the fungus was started after 2-3 days after incubation at 26 ± 2 ^{0}C. The growth of the pathogen was first appeared as whitish cottony growth and later it turns to greyish white colour due to spore formation at last it changes to greenish white colour with thick mycelium on PDA medium. The maximum growth of the pathogen *i.e*., 90 mm was

reported after ten days after incubation on PDA medium. The sporulation is evident from 7th day of inoculation. All the culture characteristics of pathogen were present as mentioned in Table1.

Table 1: Characterization of the putative pathogen causing NCLB by cultural characteristics

Sl. No.	Cultural parameter	Observation
1	Colony colour (front view)	Whitish grey
2	Colony colour (rare view)	Light grey
3	Colony diameter	9 mm
4	Colony type	Fluffy raised cottony
5	Colony margin	Regular

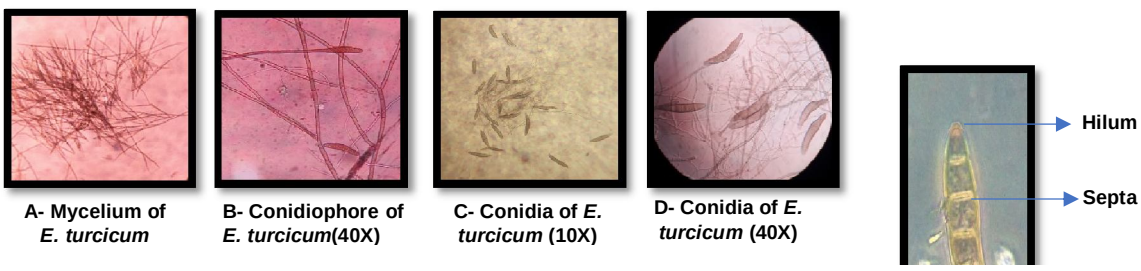
3.2.2.2 Morphological characteristics

The pathogen produced multi celled, hyaline, smooth walled, falcate conidia *i.e*., elongated spindle shaped conidia, tapering at one end and blunt at other end with presence of protruding hilum at the end that connects to conidiophore. The conidia length, conidia width and number of septa were present as mentioned in the Table 2.

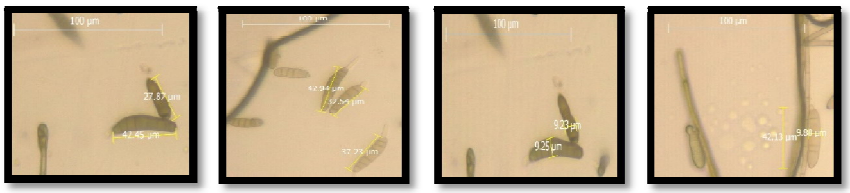
Table 2: Characterization of the putative pathogen causing NCLB by morphological characteristics

Sl. No.	Morphological parameter	Observation
1	Shape of conidia	Elongated spindle shaped
2	Colour of conidia	Hyaline
3	Conidia length	27.87- 45.01 µm
4	Conidial breadth	9.22- 9.88 µm
5	Septation	Present (5-9)
6	Hilum	Present

Based on the cultural characteristics such as colony colour, colony morphology and morphological character such as conidia length, conidia width and number of septa the isolated pathogen was identified as *E. turcicum* (Fig. 4).



A- Mycelium of *E. turcicum* **B- Conidiophore of *E. turcicum*(40X)** **C- Conidia of *E. turcicum* (10X)** **D- Conidia of *E. turcicum* (40X)**



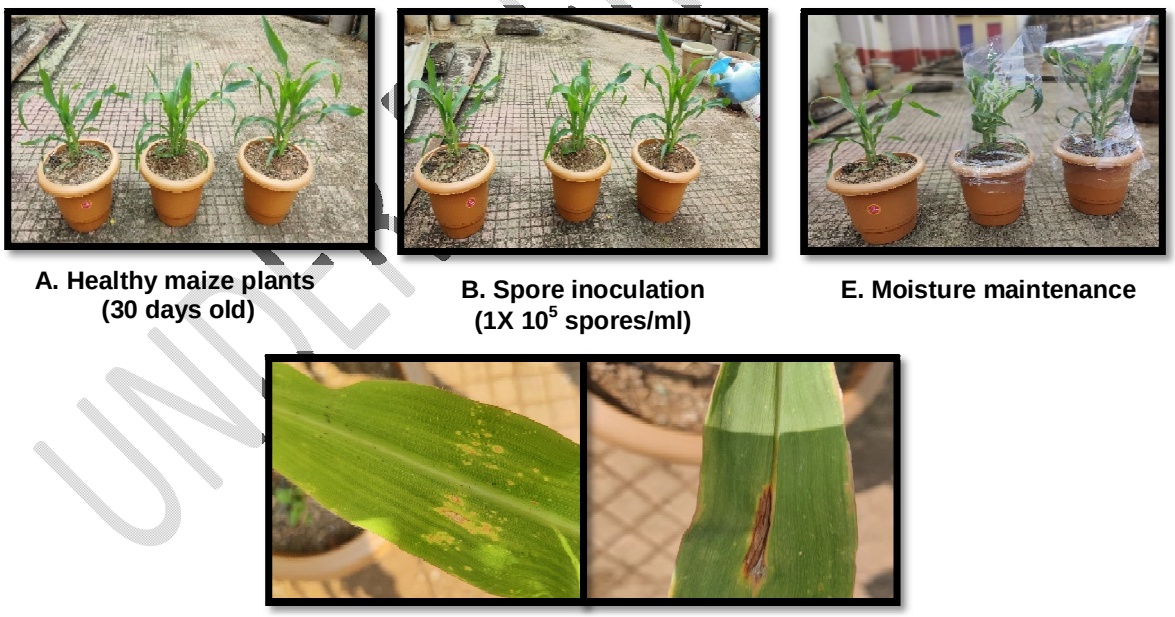
E- Conidial dimensions of *E. turcicum*

Fig.4: Microscopic characteristics of the pathogen causing northern corn leaf blight of maize

3.3 Pathogenicity of northern corn leaf blight of maize

3.3.1 Spray inoculation method

For the confirmation of the pathogenicity, the spore suspension of pathogen containing 1x10^5 spores/ml was prepared for the inoculation on the plant. Healthy maize plants of 30 days old in a pot were taken and the pathogen was inoculated, it was observed that after 5 days of inoculation small necrotic spots were found near by the leaf mid rib region and after 12 days of inoculation the necrotic blight of cigar shape was observed (Fig.5). The results revealed that the pathogen cause infection on the inoculated maize plant and the control plants shows no symptoms of the disease.



A. Healthy maize plants (30 days old)

B. Spore inoculation (1X 10^5 spores/ml)

E. Moisture maintenance

F. Observation of symptoms on artificially inoculated leaves after 5 and 12 days

Fig.5: Pathogenicity test of NCLB through spray inoculation method

3.3.2 Detached leaf assay

Pathogenicity test conducted by detached leaf assay technique on 30 days old leaves and 60 days old leaves. The results showed that, after 7 days after inoculation the symptoms on the 30 days old leaf was peculiar as necrotic blight near the mid rib region of the leaf but in 60 days old leaf the symptoms are very less and form only small spots around the mid rib (Fig.6).

Thus, the isolated pathogen was identified as *E. turcicum* which produce characteristic northern corn leaf blight of maize (*Zea mays* L.) plants.

Fig. 6 (a): Detached leaf assay (Leaves of 30 days old plant):

A. Spore inoculation ($1x\ 10^5$ spores/ml) **B. Control** **C. Symptoms on inoculated leaves after 7 days**

Fig. 6 (a): Detached leaf assay (Leaves of 60 days old plant):

A. Spore inoculation ($1x\ 10^5$ spores/ml) **B. Control** **C. Symptoms on inoculated leaves after 7 days**

Fig. 6(b): Pathogenicity test of NCLB through detached leaf assay

3.4 Molecular characterization of pathogen causing northern corn leaf blight of maize

In the current study, the DNA of the NCLB pathogen was successfully extracted and the sequence confirmation of isolated pathogen causing NCLB of maize was obtained using universal ITS oligonucleotide primer (ITS 1F and ITS 4R). Amplification of the fragment of the NCLB pathogen was seen at 574 bp length during the agarose gel electrophoresis (Fig.7). The sequence acquired from ITS rRNA PCR amplification was compared to the

sequences using the National Centre for Biotechnology Information (NCBI) BLAST program. Subsequently, the sequence was identified and deposited in NCBI GenBank to get accession number. It was found that the sequence was above 93 per cent homology with the reference sequences from the database of GenBank. A phylogenetic tree (Fig. 8) was built having a boot strap value of 98-100 per cent with pathogen and reference organism sequences.

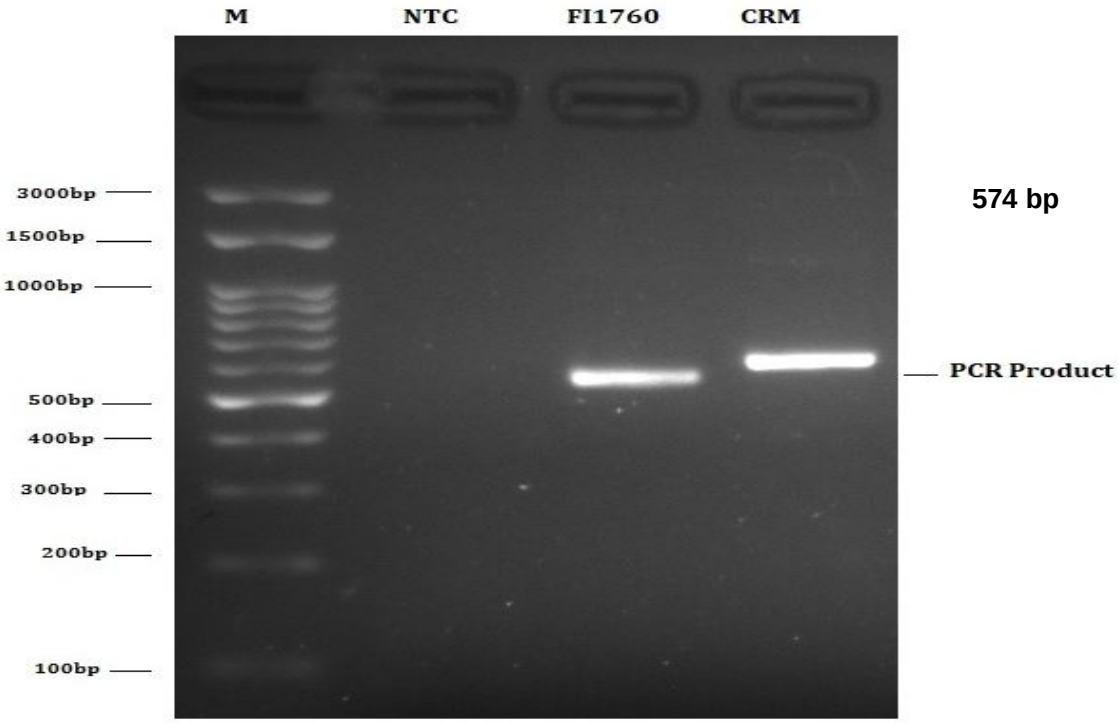


Fig. 7: PCR amplification fragments of pathogen causing NCLB; M- Ladder (1 Kb); NTC- No Template Control, FI- Fungal identification, CRM- Control Reference Material

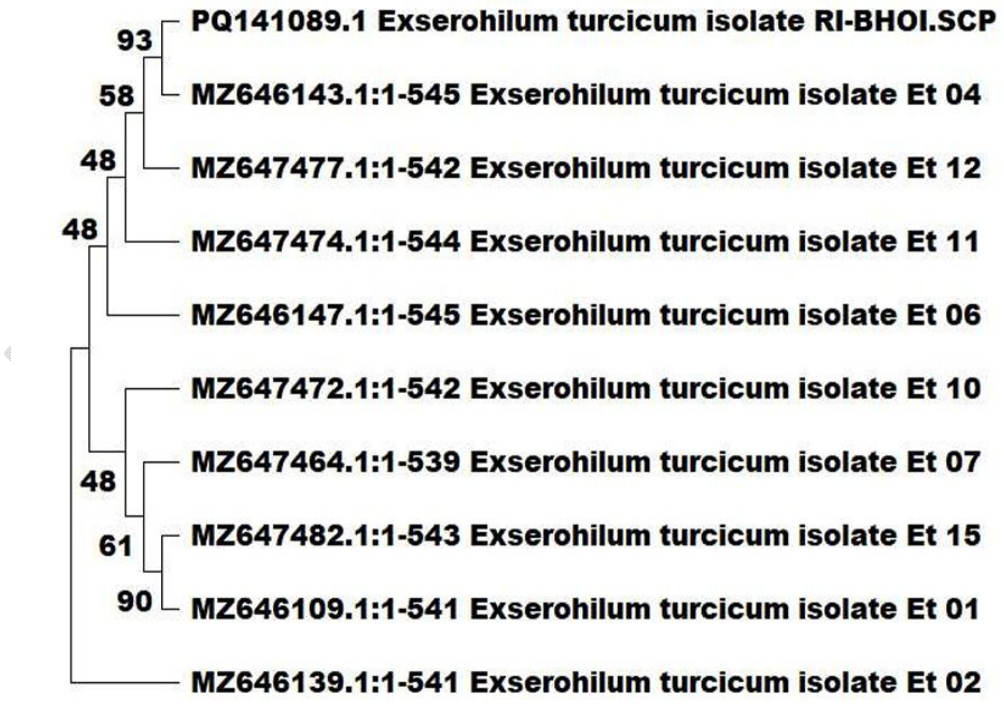


Fig. 8: Phylogenetic tree based on the maximum likelihood method analysis of the ITS rDNA region for *Exserohilumturcicum* isolate and their closest taxa(Numbers at the nodes indicate the bootstrap values from 1,000 replications)

4 DISCUSSIONS:

Among the foliar fungal diseases of maize, northern corn leaf blight (NCLB) is one of the destructive diseases which leads to a foliar and grain yield loss. The disease is very common in the maize growing regions with high humidity and continuous rainfall and the temperature range from 18-27 ^{0}C all over the world. Similarly, Savary *et al*. (2019) and Kumari *et al*. (2020) mentioned NCLB as a one of the most prominent fungal diseases of maize in the areas with high humidity and moderate temperature. Sharma (2023) founds that the development of northern leaf blight on maize throughout the growing season had inversely association with minimum temperatures and maximum temperatures while positively associated with relative humidity.

The NCLB infection of the maize plant in the field initially starts as a minute chlorotic fleck on the lowermost leaves of the plant which are near to the ground and then moves to the upper leaves of the plant. Mature symptoms were characterized by grey-green coloured elliptical or cigar-shaped lesions that are 2–7-inch length and 1-2 inch wide. As the disease progresses, the lesions start to mature and become tan coloured with distinct dark zones that are found with fungal sporulation. In the prime stage of the disease, multiple lesions can coalesce forming large irregular areas of dead tissue on the leaves. Wise (2011) and Chaudhary and Mani (2011) observed the similar symptoms of NCLB where they found cigar shaped lesions of greyish green to brown (tan) colour with a size range from 4 to 20 cm long and 1 to 5 cm wide. Singh *et al*. (2012) mentioned that the spores of the pathogen develop abundantly on either side of the spot. Heavily infected field presents a scorched appearance. Similarly, Saeed *etal*. (2023) also describes the disease moved upwards until maturity, destroying vast amounts of photosynthetically active leaf area, giving the plant a burned or burnt look and causing premature leaf death.

The maize leaves with NCLB disease symptoms were gathered from the three selected maize growing places *viz*., CPGS-AS (Umiam), ICAR RC for NEH region (Umiam), College of Agriculture (Kyrdemkulai) in Ri-bhoi district, Meghalaya. It was found that the disease was severe in CPGS-AS (Umiam) and ICAR RC for NEH region (Umiam) than in College of Agriculture (Kyrdemkulai). Similarly, Malakar (2023) reported that the disease incidence of NCLB was increasing year by year in Ri-bhoi district as the environmental conditions became more favourable. According to Ullstrup (1966), the occurrence and intensity of NCLB in maize exhibit annual and regional variations primarily depends on the environmental factors such as temperature and rain fall.

The pathogen that causes NCLB of maize was isolated from infected maize leaves exhibiting the typical 'cigar shaped' lesion symptoms of NCLB which were collected from the research maize fields of CPGS-AS (Umiam), ICAR RC for NEH region (Umiam), College of

Agriculture (Kyrdemkulai) in Ri-bhoi district, Meghalaya. The isolation of the pathogen was done through two methods *i.e*., spore germination method and hyphal tip inoculation method. Similar type of results was obtained by Muiru*et al*. (2010) when they isolated the NCLB pathogen through leaf bit inoculation method from different parts of Kenya, Germany and Austria. Similarly, Navarro *et al*. (2021) isolated the NCLB pathogen through spore germination method from maize cropping areas of Argentina and Brazil. The findings also supported by Turgay *et al*. (2020) where they isolated the NCLB pathogen through spore germination method. After isolation of the pathogen, it was purified by hyphal tip isolation and the culture of the pathogen which is purified was stored in the PDA slants at 4 ^{0}C in refrigerator. Meghana *et al*. (2023) also finds the similar results as they isolated *E. turcicum* through hyphal tip isolation method and stored at 5 ^{0}C in refrigerator in PDA slants.

The pathogen isolated from the NCLB infected maize leaves was characterized as *Exserohilumturcicum* through the cultural, morphological and microscopic observations. The mycelial growth of the fungi on the PDA medium was observed as light grey colour on the front view with cottony appearance. Similarly, Rajula *et al*. (2017) reported that the colony colour of isolates of *E. turcicum* range from grey to dark grey with cottony appearance. Wathaneeyawech*et al*. (2015) also finds the similar results and mentioned that the appearance of the isolates as whitish grey colour with thick mycelium.

From the morphological observations, the mycelium and conidia of *E. turcicum* is appeared in hyaline in colour with predominant septation and the conidial length ranges from 27.87- 45.01 µm, conidia breadth ranges from 9.22- 9.88 µm and the septation range from 5-9 number. Similarly, Sethy *etal*. (2023) from Manipur mentioned that the conidial length ranged from 91.70 to 57.35 µm, conidial width ranged from 18. 01 to 11. 09 µmand no. of septa ranged from 4 to 6. Banerjee and Nath (2023) found similar results and they mentioned that the septa number range from 5-9. Conidial length ranged from 52.94 µm to 144.12 µm as mentioned by Anwer*et al*. (2022). The shape of the conidia was observed as elongated-spindle shaped with projecting hilum. Similarly, the shape of the conidia of *E. turcicum* was found as elongated and spindle-shaped with projecting hilum by Anwer*et al*. (2022). The cultural and morphological characters of the pathogen in the current investigation was also identified similar as mentioned by Abebe and Singburaudom (2006) and Bankole *et al*. (2023).

The pathogenicity of the NCLB of maize to detect the symptoms and to validate the Koch's postulates. The maize plants of 30 days old were infected with a spore suspension of 10^5 spores/ml. The early symptoms of small necrotic spots were found near the leaf mid rib region after 5 days of inoculation, the typical cigar shaped lesion on the leaf was found after

12 days of inoculation, the control plants don't show any symptoms of NCLB. Similarly, Onwunali and Mabagala (2022) and Aghav*et al*. (2023) conducted the pathogenicity through spray inoculation method on the maize plants to validate the Koch's postulates and found the similar results. The present report also supported by Patil *et al*. (2022) as they also found the similar results of pathogenicity after 14 days after inoculation and Jindal *et al*. (2019) also observed the typical symptoms of NCLB after 7-14 days after inoculation. Balass and Levy (1984) observed the similar results on the maize plants when they inoculated the spores of *E. turcicum*.

To observe the pathogenicity of *E. turcicum* through detached leaf assay method spore suspension with concentration of 1x 10^5 spores/ml was sprayed on detached maize leaves of 30 days and 60 days old and it was observed that the severe symptoms like cigar shaped lesions were observed on 30 days old maize leaves when compared to 60 days old maize leaves where less severe symptoms like small necrotic spots were found after 7 days of inoculation. Similar findings were obtained by Levy and Cohen (1983) when they inoculated the pathogen on the detached leaves of maize, they observed lesions on the foliage and spores microscopically. Muiru*et al*. (2008) additionally confirmed and prove the casual pathogen of NCLB of maize by detached leaf assay and they observed the similar type of symptoms like small necrotic spots earlier and typical cigar shaped lesions at later stages of inoculation.

The molecular characterisation of the NCLB pathogen was performed using universal primers (ITS 1F and ITS 4R), and the pathogen was identified as *Exserohilumturcicum*. The present reports are in compliance with those of Zahara *et al*. (2022) who performed the DNA sequencing and PCR amplification of NCLB pathogen obtained from maize using ITS1/ITS4 primers and they observe the PCR amplification at 600 bp and the isolated pathogen was confirmed as *E. turcicum*. Similarly, Ferdinandez*et al.* (2020) also extracted the genomic DNA of pathogen of rice, early barnyard grass and identified the pathogen morphologically as *Exserohilum* spp., through PCR amplification using ITS1 and ITS4 and phylogenetic analysis by maximum likelihood analysis, they confirmed the pathogen as *Exserohilum* spp. Félix-Gastélum*et al*. (2018) also did the molecular characterisation of ITS rDNA region of NCLB pathogen from maize using ITS1 and ITS4 universal primers and the sequences were compared in NCBI using BLAST-N software and Megablast algorithm and confirmed the pathogen as *E. turcicum*. Similarly, Hernãndez-Restrepo *et al*. (2018) characterized the NCLB pathogen molecularly and they found 11 isolates of *Exserohilum* and Kutawa*etal*. (2017) similarly found 5 isolates of *Setosphaeriaturcica* (anamorph *E. turcicum*) by using the universal primers. Heidarian*et al*. (2018) also amplified the ITS1+5.8S+ITS2 rDNA region of Exserohilum isolates using ITS1 and ITS4 universal primers, they analyzed the sequences in

NCBI BLAST searches and they identified the isolates as *E. monoceras*, *E. turcicum*and *E. rostratum.*

CONCLUSION:

The current study sheds light on the prevalence of *Exserohilumturcicum*, the causal agent of Northern Corn Leaf Blight (NCLB), in the Ri-Bhoi district of Meghalaya. A comprehensive investigation encompassing the isolation and characterization of *E. turcicum* from the research fields of the district was conducted. This study provides valuable insights into the morphological, molecular, and pathogenicity of the *E. turcicum* population in the region. It also highlights the potential for the emergence of new virulent strains, emphasizing the need for continuous monitoring and the development of effective resistance strategies to manage NCLB effectively. The data generated in this study can serve as a reference for future research on the epidemiology and management of NCLB in Meghalaya, contributing to the overall understanding and control of this important maize disease.

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