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RESEARCH ARTICLE

# Variability in Bamboo Blight Etiology within *Bambusa tulda* across agroclimatic zones of Assam

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**Abstract**: The impacts of bamboo blight are both economic and ecological. It affects bamboo cultivation. The disease weakens bamboo stands, making them vulnerable to other stressors and changing local biodiversity dynamics. *Bambusa tulda* Roxb., the main bamboo in Assam, is widely cultivated and grows well in different ecological niches. The current study investigated the occurrence and cause variation of bamboo blight in *B. tulda* across the six agro-climatic zones of Assam. Fungal isolates cultured from diseased culms were characterized through morphological and molecular analyses. Internal Transcribed Spacer (ITS) sequencing identified the pathogens as *Fusarium fujikuroi* (NR_111889.1), *F. bambusarum* (NR_1761461.1), *F. pseudonygamai* (NR_137162.1), *F. circinatum* (NR_120263.1), and *F. oxysporum* f. sp. *circeris* (MK_752682.1), corresponding to the different zones. Variations in symptoms of *B. tulda* in response to the blight across zones suggest possible interactions between genotypes and pathogens, influenced by environmental factors. This is the first detailed account of the molecular diversity of *Fusarium* species linked to bamboo blight in Assam. It provides essential data for developing sustainable disease management strategies for its cultivation.

*Keywords:* Agro-climatic zones, blight, *Fusarium,* intra-specific diversity, etiology

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## Introduction

Bamboo is a crucial part of the vegetation in North East India. According to the Indian State of Forest Report (IFSR, 2023), the total bamboo bearing area in India is estimated at 154,670 sq. km, with substantial area in the North Eastern states. Bamboo is known for regulating soil erosion, maintaining soil quality, and restoring mined sites. It has become an essential plant for various afforestation and regeneration efforts.

*Bambusa tulda* Roxb. (Indian timber bamboo) is important ecologically, economically, and culturally in North East India. It is found widely in Assam, Meghalaya, Nagaland, Tripura, and Arunachal Pradesh. This species thrives in different habitats, including riverbanks, plains, and hilly slopes. The culms are tall culms (20–30 m high, 15-30 cm thick) with dense canopies of lanceolate leaves and a deep rhizome that enhances soil stability and nutrient uptake. Ecologically, *B. tulda* prevents soil erosion, regulates water flow, and provides food and habitat for diverse fauna, while also serving as an important raw material for local industries and traditional crafts.

In spite of its hardiness and adaptability, the species is affected by different diseases and pests that affect various organs like shoots, culms, roots, leaves, flowers and kernels, in both natural habitat and in managed environments such as plantations, nurseries, and seed storage (Mohanan and Jyoti, 1994; Mohanan 1997). Fungi are the major cause of bamboo diseases and at present, more than 580 fungi and five bacteria, and three phytoplasmas, one

bacteria-like organism and two viruses have been reported as pathogens (Mohanan and Liese, 1990; Mohanan, 2017). Among these fungal diseases, blight and rot are the most common which cause extensive damages to the emerging and growing culms of bamboo stands (Mohanan, 1997).

Assam boasts a terrain that ranges from the plains to the hills and mountains, which divides it into six agro-climatic zones viz., Upper, Central and Lower Brahmaputra Valleys, Barak Valley, Hill Zone and North Bank Plain. Widely cultivated in the State, *Bambusa tulda* is susceptible to blight. The disease is characterised by truncated culms showing prominent dieback with the downward progression of the disease. This affects bamboo cultivation and raw material demand of industries such as construction, handicrafts, and paper production.

Despite numerous reports on bamboo diseases from various parts of India, detailed studies on the molecular diversity of blight-causing fungi in *Bambusa tulda* across the different agro-climatic zones of Assam remain limited. Most existing investigations focus on pathogen identification without examining regional variation or genetic differentiation. The present study fills this gap by employing morphological and ITS-based molecular characterization to dissect the diversity and distribution of fungal pathogens associated with bamboo blight in Assam. This paper, therefore, focuses on characterizing the causal organism of bamboo blight in *Bambusa tulda* across the agro-climatic zones of Assam.

## Materials & methods

### Sample Collection and Isolation of Causal Organism

The survey was conducted by following roving survey method in the six agro-climatic zones of Assam viz. Upper Brahmaputra Valley, Lower Brahmaputra Valley, Central Brahmaputra Valley, North Bank Plain, Hill Zone and Barak Valley (Fig 1.). The samples were collected from the *Bambusa*

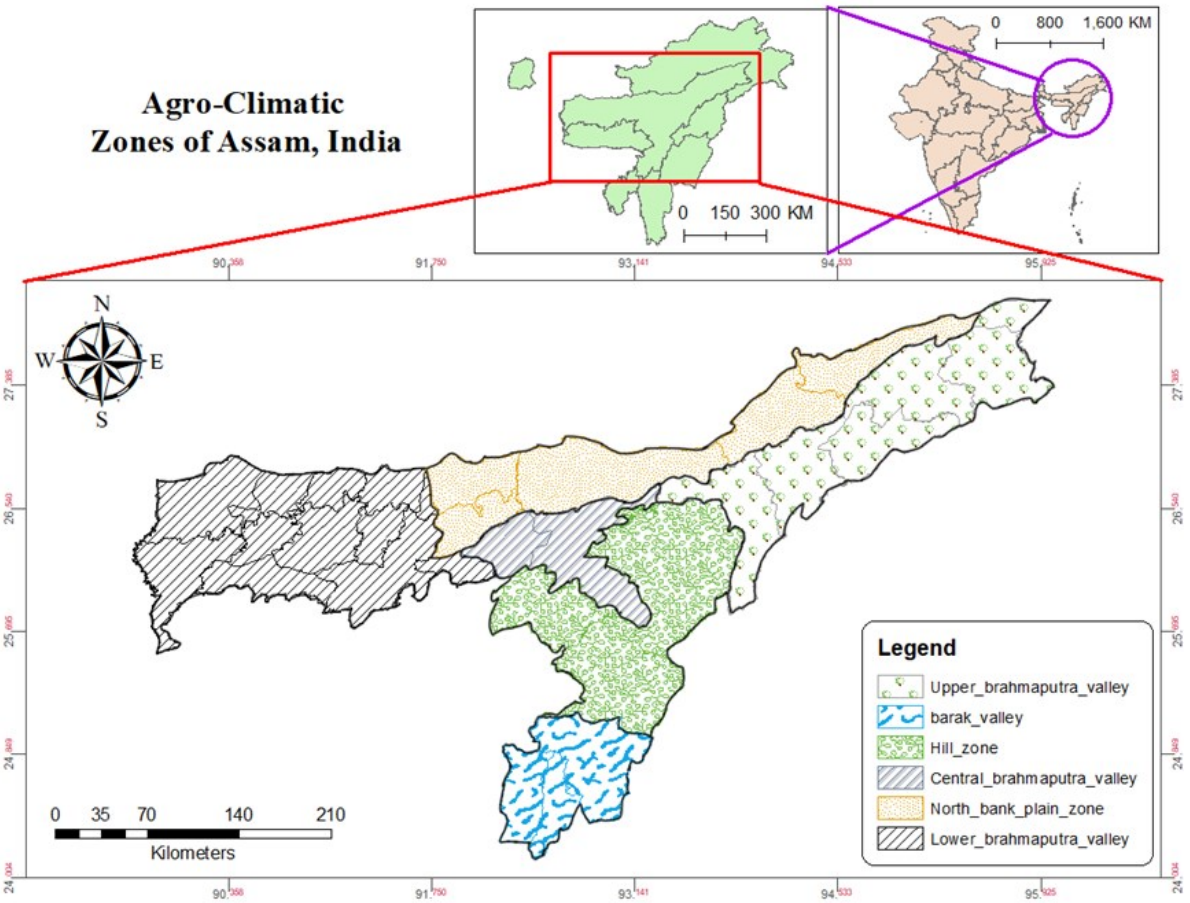


**Fig 1.** Map of surveyed areas for blight in *Bambusa tulda*

*tulda* plants showing the blight symptoms such as the truncated culms with dieback, and partial necrosis which is seen underneath the culm sheaths.

The collected diseased samples were cut into smaller pieces, washed with sterile water and then sterilised with 0.04 % Sodium hypochlorite. These sterilised samples were then transferred into petri plates containing PDA media. A quantity of 5ml of streptomycin was also added to 250 ml of PDA media to inhibit bacterial growth. The plates were then incubated at 24 ± 1°C for ten days and were examined periodically.

The percent incidence of the disease across the different agro-climatic zones were also calculated using the following formula (Mohanan, 1994):

Disease Incidence (%) = (Number of diseased plants / Total number of plants observed) × 100

**Morphological Identification of Causal Organism**

Ten-day-old pure cultures were used for detailed morphological studies. Using an inoculating needle, bits of mycelial growth were mounted in Lacto Phenol Cotton Blue stain (LPCB) and sealed with DPX mountant. The prepared mounts were then observed under a compound microscope with a photographic attachment. For authentic identification and confirmation, the fungal cultures were referred to National Centre for Microbial Resource, Pune, Maharashtra.

**Pathogenicity Test**

Test for pathogenic association of isolated fungus was done on *B. tulda* seedlings and was confirmed through Koch's postulates (Koch, 1882). For the pathogenicity test, 28 six-month-old *Bambusa tulda* seedlings, approximately 1–1.5 m in height, were used, with four seedlings serving as uninoculated controls. The remaining seedlings were inoculated with fungal isolates obtained from the six agro-climatic zones of Assam. The experiment was conducted in a greenhouse under controlled environmental conditions - 25–28°C, 70–80% relative humidity, and natural photoperiod. Inoculations were performed by inserting a 2 mm diameter plug of mycelium-containing agar into the third culm sheath from the apex using sterile forceps, without causing injury, and the site was covered with a piece of moist absorbent cotton. Both inoculated and control seedlings were enclosed in moistened polythene bags for four days and monitored periodically for symptom development. Re-isolation of the fungus from artificially infected seedlings was done on PDA media.

**Molecular Characterization and Phylogenetic Analysis**

Molecular characterization was performed using the internal transcribed spacer (ITS) region of ribosomal DNA. Genomic DNA was extracted as per Doyle and Doyle (1990) from fungal isolates and the ITS region amplified using universal primers ITS1 and ITS4 following White *et al.* (1990). PCR amplification was performed in a 25 µL reaction mixture, and the products were purified using a PCR purification kit (Qiagen, Germany). Sequencing was performed on an automated DNA sequencer (Applied Biosystems 3730). The obtained sequences were aligned using CLUSTAL W, and phylogenetic analysis was conducted using the Maximum Likelihood method in MEGA v11 (Tamura *et al*., 2021). Bootstrap analysis was performed with 1000 replicates. The resulting sequences were compared with those available in GenBank using BLAST to determine species identity (Boratyn *et al*., 2013).

**RESULTS**

**Etiology of Blight in *B. tulda***

The blight in *B. tulda* appears mostly in the first year, when the culms reach a height of 5-6 metres. The symptom first appears as brown necrotic patches on culm sheaths, which cover the internodes. The affected sheaths are loosely attached and, upon removal, exhibit decay symptoms. These apical sheaths then die and the apical portion curves downwards. Dieback starts at the top and progresses downwards. Just below the dieback branch, partial necrosis can be seen, which often stays hidden beneath the culm sheaths. The "dieback" region sometimes harbours various insects such as ants, earwigs etc. The symptomology of the blight is illustrated in Fig 2.

The most characteristic feature of the blight marked by truncated culms showing prominent dieback was seen in all the agro-climatic zones, but certain variations in the symptoms were also seen in

all the agro-climatic zones, but certain variations in the symptoms were also seen in different agro-climatic zones of Assam. For instance, in the North Bank Plain, the blight was also seen in the branches of the dieback culm. Similarly, the blighted culms were accompanied by chlorosis in North Bank Plain, Barak Valley, as well as in Upper Brahmaputra Valley. Yellow stripes were observed in the diseased culms from Hill Zone. Chlorosis of nodes was also found in diseased bamboos of Central Brahmaputra Valley. The variations in symptoms are shown in Fig 3.

Based on the percentage incidence of blight disease, a marked variation was observed across the agroclimatic zones of Assam. The highest incidence was recorded in the Upper Brahmaputra Valley (42.97%), followed by the North Bank Plain (39.01%) and the Hill Zone (33.67%). Comparatively lower incidences were observed in the Lower Brahmaputra Valley (25.45%) and Barak Valley (23.00%), while the Central Brahmaputra Valley exhibited the minimum incidence (20.01%) as shown in Fig 4. This zonal variation in disease incidence suggests that environmental and agroecological factors prevailing in the Upper Brahmaputra Valley may be more conducive to blight development and spread compared to other regions.


**Fig 2.** Symptomology of the bamboo blight in *B. tulda*; A. Blighted culm, B. Arc formation of the blighted culm, C. Downward progression of the disease, & D. Dead blighted culm

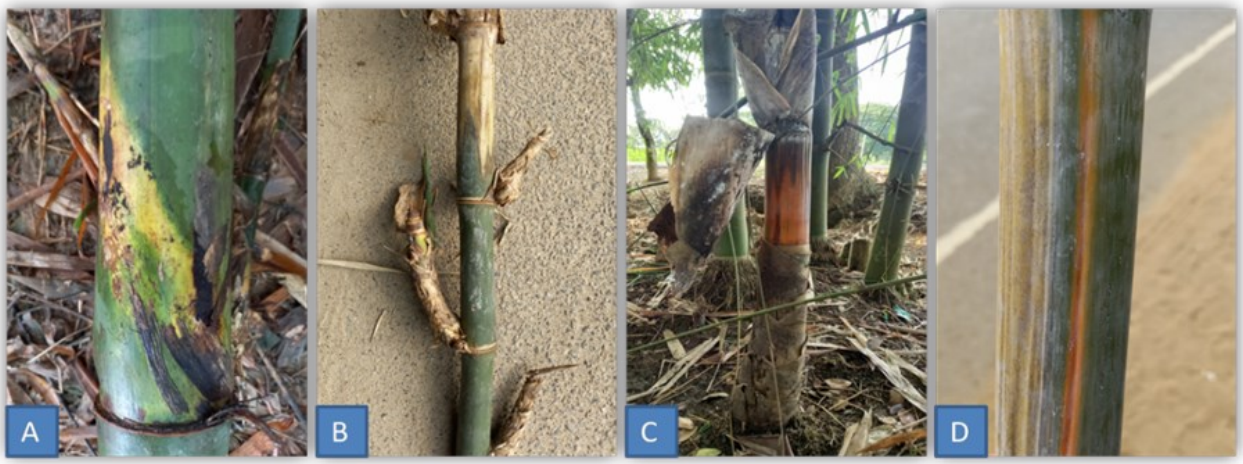


**Fig 3.** Variation in symptoms, A). Chlorosis of the blighted culm, B). Blighted branches seen in North Bank Plain, C). Chlorosis of the node in the Central Brahmaputra Valley & D). Yellow stripes found in diseased culm in Hill Zone

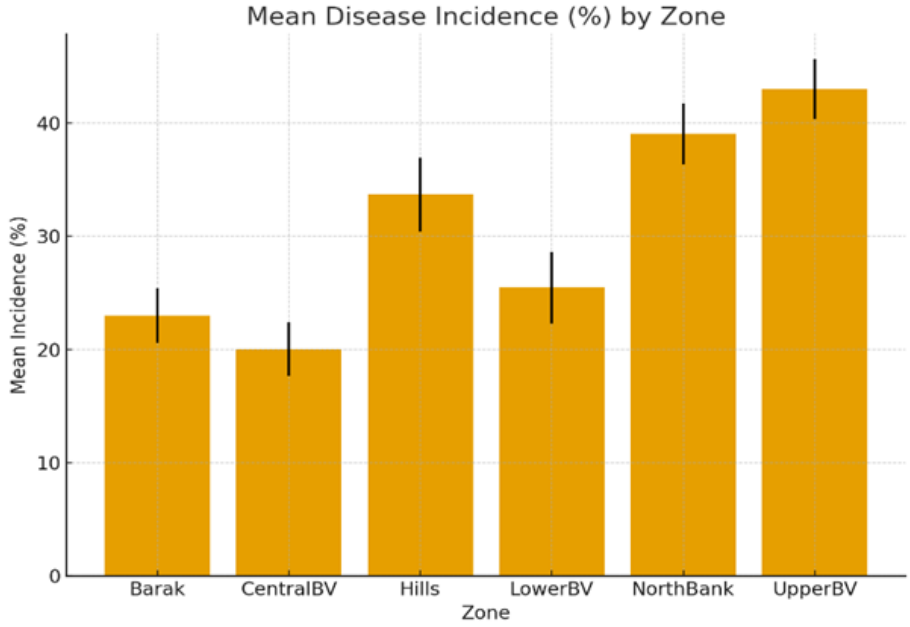


**Fig 4.** Percentage incidence of bamboo blight across different agro-climatic zones of Assam

### Identification of Causal Organism

The isolates from the different agro-climatic zones were preliminary identified based on the colony morphology. The colonies from all the agro-climatic zones were morphologically similar. The colonies were characterized by fluffy to cottony texture, circular shape and off-white colour with pink and carmine pigmentation. Macroconidia were mostly long, slender, sickle-shaped, septate. Microconidia appeared as small, oval shaped spores. The details are at Fig 5 and Fig 6.

From the morphological examination, the causal organism isolated from the six agro-climatic zones was identified as belonging to the genus *Fusarium*. For further identification, confirmation, and for molecular characterization, the fungal cultures were referred to National Centre for Microbial Resource (NCMR), Pune, Maharashtra, India. The results based on ITS sequencing revealed that the fungal pathogens from the agro-climatic zones of North Bank Plain, Hill, Upper Brahmaputra Valley, Central Brahmaputra Valley, Lower Brahmaputra Valley and Barak Valley were *Fusarium fujikuroi* (NR_111889.1), *F. bambusarum* (NR_1761461.1), *F. bambusarum* (NR_1761461.1), *F. pseudonygamai* (NR_137162.1), *F. circinatum* (NR_120263.1) and *F. oxysporum f. sp. circeris* (MK_752682.1) respectively.

### Pathogenicity Test

Upon inoculation with the fungal pathogen, blight symptoms developed in 24 *B. tulda* seedlings inoculated with six *Fusarium* isolates (one from each agro-climatic zone), with four replicate seedlings per isolate, while the four uninoculated control seedlings remained symptom less throughout the observation period. The symptoms of bamboo blight in seedlings became evident within 7–10 days. Initially, small lesions appeared at the stem and leaf sheath junctions. As the infection advanced, lesions coalesced, leading to blighting of the leaf sheaths and partial wilting of the affected shoots. With the progress of infection, the dieback symptoms could be seen in the infected seedlings as shown in Fig 7. The control seedlings remained healthy. Re-isolation from the infected seedling samples yielded the five different species mentioned above namely *Fusarium fujikuroi, F. bambusarum, F. pseudonygamai, F. circinatum and F. oxysporum f. sp. circeris.*

### Phylogenetic Analysis

The molecular characterization of the pathogen revealed that the causal organisms of bamboo blight in *B. tulda* are fungi of the family Nectriaceae and genus *Fusarium*. Interestingly, the species are different in each agro-climatic zone with the exception in Hill Zone and Upper Brahmaputra

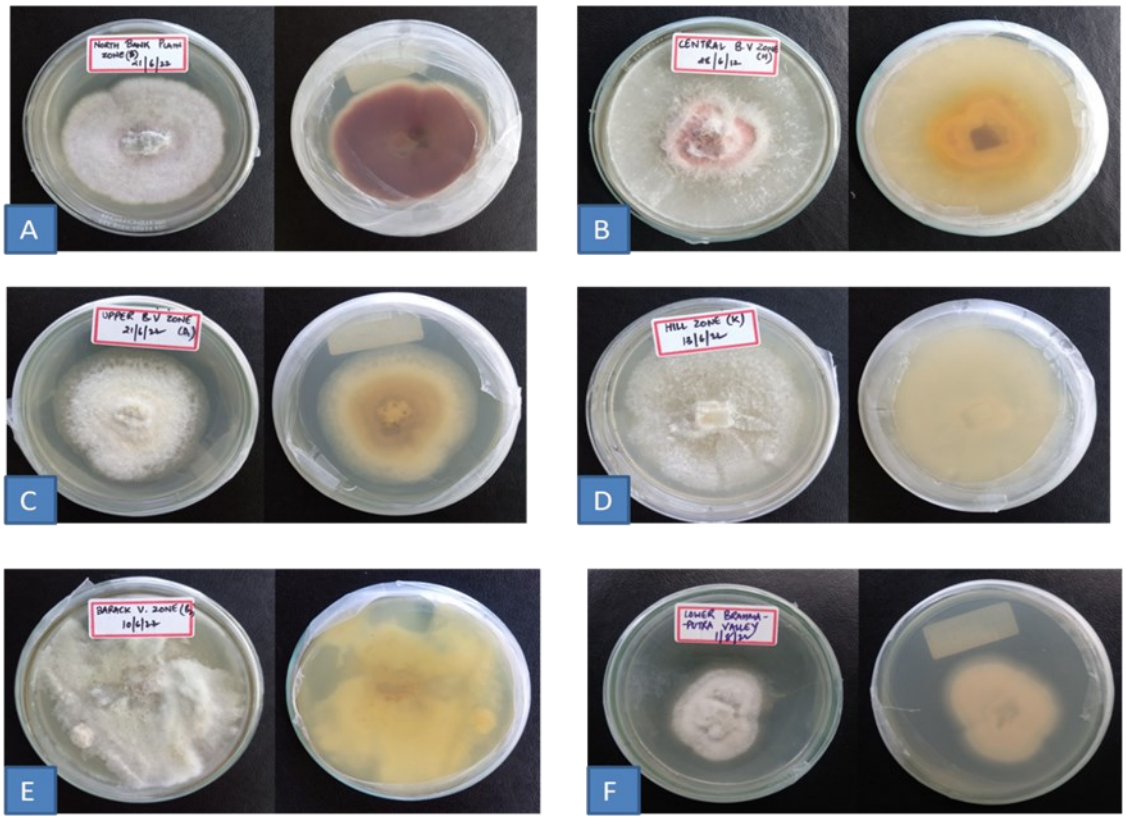


**Fig 5.** Causal organism identified from different agroclimatic zones viz. A) *Fusarium fujikuroi* (NR_111889.1), B) *F. pseudonygamai* (NR_137162.1), C & D) *F. bambusarum* (NR_1761461.1), E) *F. oxysporum f. sp. circeris* (MK_752682.1), F) *F. circinatum* (NR_120263.1)

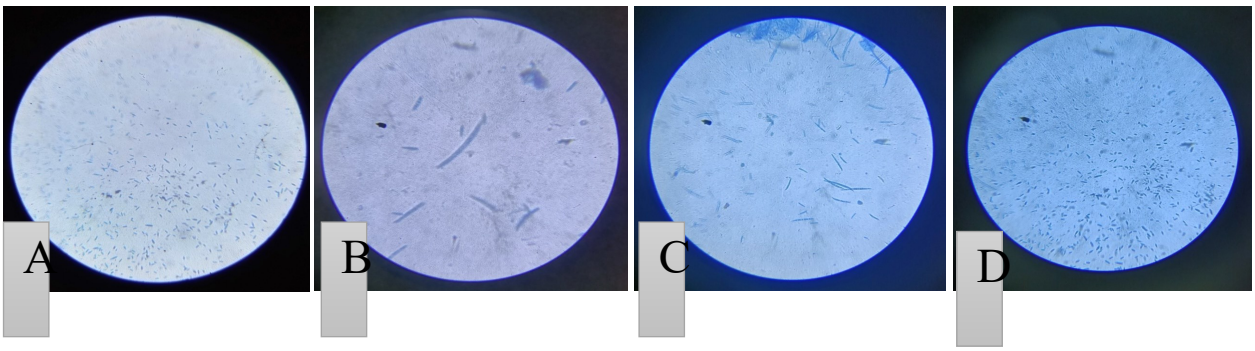


**Fig 6.** Microscopic images of macroconidia and microconidia of isolated *Fusarium* species

Valley, where they belong to the same species. The phylogenetic analysis also revealed that the pathogens belong to two clades. The phylogentic tree is in Fig 8. Clade 1 consists of *F. bambusarum* (NR_1761461.1), *F. circinatum* (NR_120263.1), *F. pseudonygamai* (NR_137162.1 and *Fusarium fujikuroi* (NR_111889.1) and Clade 2 consists of F. *oxysporum f. sp. circeris* (MK_752682.1). It can be observed that *F. bambusarum* was the common pathogen in both Hill Zone and Upper Brahmaputra Valley, which might be due to the proximity of their geographical regions. Similarly, pathogens of Lower Brahmaputra Valley and Central Brahmaputra Valley viz., *F. circinatum* and *F. pseudonygamai* are closely related to each other. The slight variations in symptomology across different agro-climatic zones can be attributed to the variation in the species.

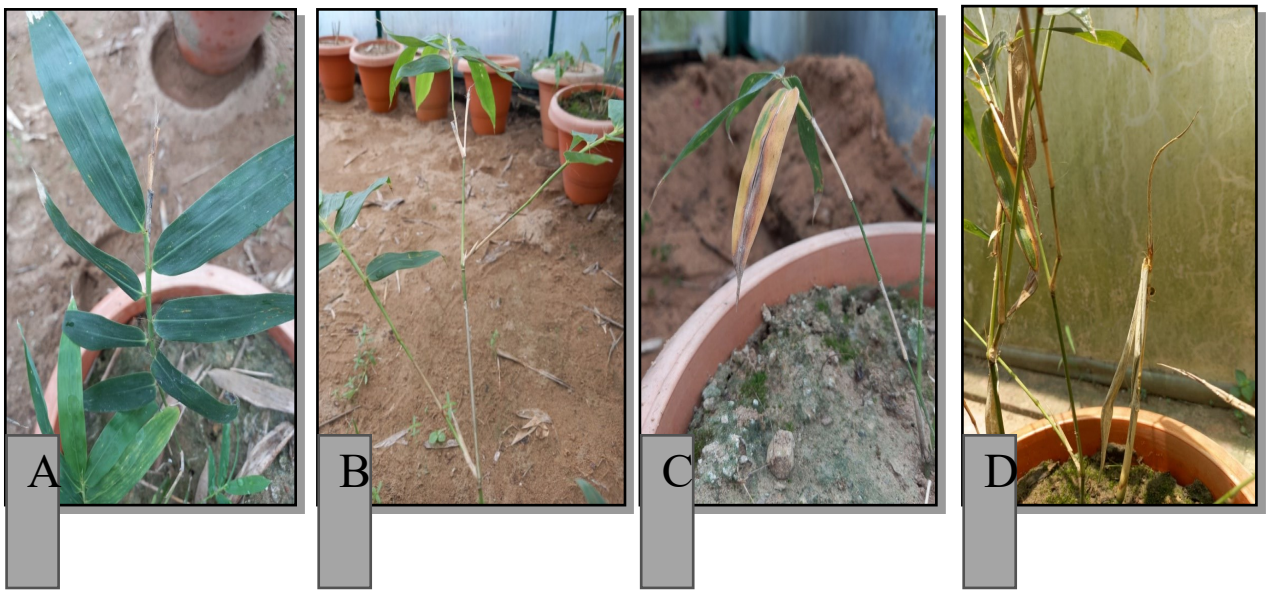


**Fig 7.** Pathogenicity Test: A) Intiation of blight by discolouration of tip of the leaves, B) The blight showing downward progression, C) Wilting of the leaves and D) Complete dieback of the seedling

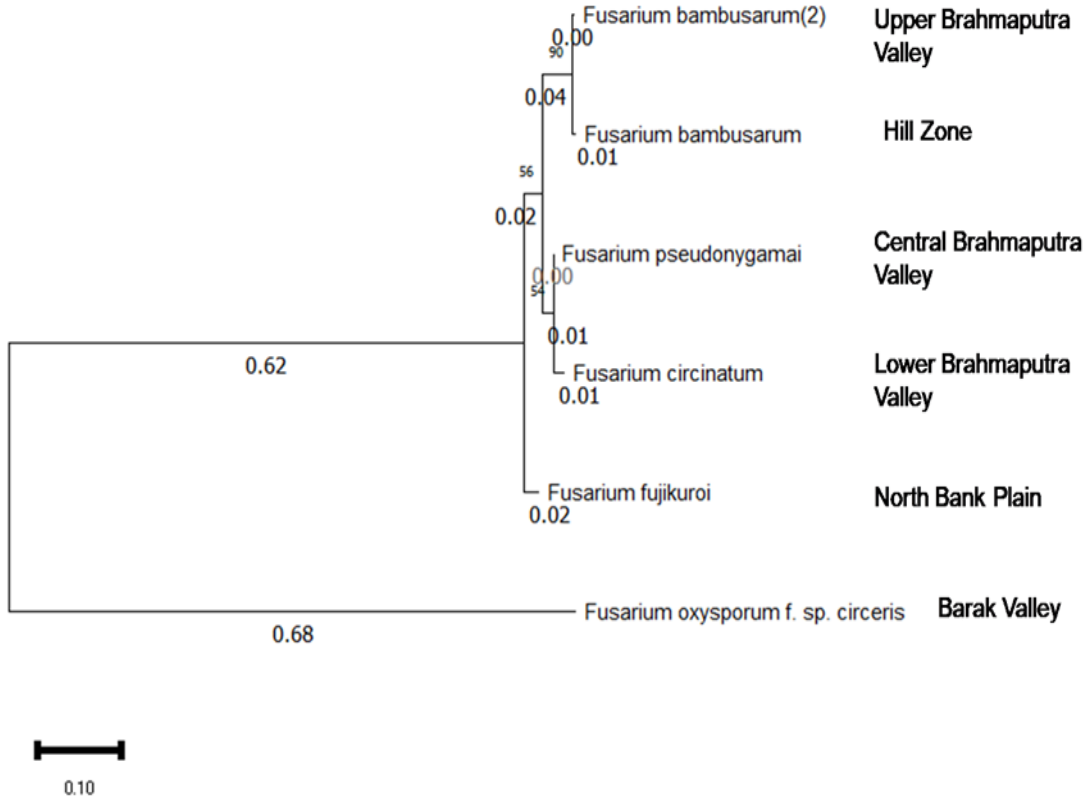


**Fig 8.** Phlogentic tree of the pathogens from different regions.

## Discussion

The incidence of blight disease in *Bambusa tulda* exhibited marked variation across the agroclimatic zones of Assam. The highest disease incidence was recorded in the Upper Brahmaputra Valley (42.97%), followed by the North Bank Plain (39.01%) and the Hill Zone (33.67%). Intermediate incidences were observed in the Lower Brahmaputra Valley (25.45%) and Barak Valley (23.00%), while the Central Brahmaputra Valley showed the lowest incidence (20.01%). The highest severity of bamboo blight in Upper Brahmaputra valley is likely due to high humidity with annual rainfall of ~2500-3000 mm. In contrast the Central Brahmaputra Valley receive comparatively lower annual rainfall ~1800-2300 mm. These conditions in Upper Brahmaputra Valley are more favourable for growth and disease progression of *Fusarium* species. This variation shows that local environmental and agroecological conditions, especially in the Upper Brahmaputra Valley, may promote blight development and allow pathogens to spread more easily than in other areas. These patterns could help us investigate the underlying diversity of the pathogens, how symptoms appear, and their geographical relationships in future studies.

Molecular characterization based on ITS identified that blight was caused by different *Fusarium* species across the zones. *F. fujikuroi* was found in the North Bank Plain; *F. bambusarum* in the Hill Zone and Upper Brahmaputra Valley; *F. pseudonygamai* in the Central Brahmaputra Valley; *F. circinatum* in the Lower Brahmaputra Valley; and *F. oxysporum f. sp. ciceris* in the Barak Valley. Although the overall symptoms of the disease were mostly similar, minor differences were noted. These included chlorosis of nodes in the Central Brahmaputra Valley, yellow striping in the Hill Zone, and blighted branches in the North Bank Plain. These variations likely reflect differences at the species level in *Fusarium* and the influence of environmental factors. Phylogenetic analysis showed that closely related *Fusarium* species grouped in nearby areas. For example, *F. pseudonygamai* and *F. circinatum* were collected from the Central and Lower Brahmaputra Valleys, while *F. bambusarum* was found in both the Upper Brahmaputra Valley and Hill Zone. In contrast, *F. oxysporum* from the Barak Valley was distantly related to other pathogens, likely because it is geographically isolated from the Brahmaputra basin. The phylogenetic tree indicates that geographic barriers and environmental factors significantly influence pathogen distribution and diversity. The results indicate that bamboo blight in *B. tulda* is caused by *Fusarium* species complex rather than by a single pathogen, but environmental selection likely allows one species to dominate in each agro climatic zone. This study is the first in Assam to document the various Fusarium species in *Bambusa tulda* across different agroclimatic zones, along with associated differences in symptom expression and causes. Previous reports identified only single species *F. semitectum* in Nagaland (Gogoi *et al*., 2013) and *F. udum* in plantations (Borah, 2006). This study provides a solid foundation for understanding the complexity of pathogen populations in the region. The spatial distribution of pathogens and slight differences in symptom expression are important for evolution and indicate the species ecological adaptation. Similar findings in yam (*Dioscorea polystachya*) noted that multiple *Fusarium* species showed significant genetic differences among geographically separated populations (Dongzhen *et al*., 2020).

Overall, these results highlight how pathogen diversity, environmental factors, and geographic separation influence blight expression in *B. tulda.* The study offers valuable insights into the spatial ecology, evolution, and causes of bamboo blight in Assam. This opens opportunities for future research on plant-pathogen interactions in different agroecological conditions (Franic *et al*., 2023; Treindl *et al*., 2023).

As a group that is both economically damaging and diverse, future research should expand to map *Fusarium* diversity and explore host-pathogen interactions to identify resistant *B. tulda* genotypes. Molecular and population genetic studies could clarify its evolution and local adaptation. Since *B. tulda* is widely cultivated, developing integrated management strategies, including biological control, targeted fungicides, and improved farming practices, would support its sustainable cultivation.

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In preparing this manuscript, the author(s) utilized Grammarly for language editing and improving the clarity of the manuscript. The authors confirm that the manuscript represents their original work and that they take full responsibility for the accuracy, integrity, originality, and scientific content of the article.

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