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Occurrence and characterization of *Bipolaris setariae* associated with leaf blight of browntop millet (*Brachiaria ramosa*) in India

Gutha Venkata Ramesh[1] | **Kaki Boraiah Palanna**[2] | **Hargi Devappa Vinaykumar**[2] | **Arunkumar**[1] | **Prasanna S. Koti**[3] | **Hosapura Shekhararaju Mahesha**[4] | **Thathigowdara Enkeswarappa Nagaraja**[2] | **Vilas A. Tonapi**[5] | **B. Jeevan**[6]

[1]Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bengaluru, India

[2]Project Coordinating Unit, ICAR-AICRP on Small Millets, UAS, GKVK, Bengaluru, India

[3]The University of Trans-Disciplinary Health Sciences and Technology, Bengaluru, India

[4]ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

[5]ICAR-Indian Institute of Millets Research, Hyderabad, India

[6]ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, India

Correspondence
Kaki Boraiah Palanna, Project Coordinating Unit, ICAR-AICRP on Small Millets, UAS, GKVK, Bengaluru, Karnataka, 560065, India.
Email: kbpalanna@gmail.com

B. Jeevan, Crop Protection Division, ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora, Uttarakhand, 263601, India.
Emails: jeevan.b@icar.gov.in; jeevan.bscag@gmail.com

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Abstract

A new leaf blight disease of browntop millet (*Brachiaria ramosa*) was noticed during rainy season (*Kharif*) 2018 at small millet experimental field, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra (GKVK), Bengaluru, India. To assess the disease severity, an intensive roving survey was conducted during the 2019 cropping season. Based on the morphological characterization, the causal agent of leaf blight disease was identified as *Bipolaris* spp. Further sequencing and combined gene analysis of ITS (internal transcribed spacer of rDNA), GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and LSU (large subunit) of all the nine isolates confirmed the pathogen as *B. setariae*. Pathogenicity study showed that all the isolates were pathogenic and caused leaf blight symptoms on browntop millet. The *B. setariae* isolates showed marked variability with respect to disease incidence on browntop millet (cv. Dundu korale) under artificial inoculation conditions. However, the host range was limited only to browntop millet and found non-pathogenic to other six small millets examined. To our knowledge, this is the first completely described study on characterization of *B. setariae* causing leaf blight disease of browntop millet in India.

KEYWORDS

Bipolaris setariae, browntop millet, characterization, host range, leaf blight, virulence

1 | INTRODUCTION

The genus *Bipolaris* (Shoemaker, 1959), with teleomorphic state in *Cochliobolus* (Drechsler, 1934), contains many plant pathogens with a broad host range mainly in Poaceae. The pathogenic species of the genus *Bipolaris* are of great importance in various countries around the world, including India, and have been reported to cause fatal diseases in economically important crops such as rice, wheat and maize (Jeevan et al., 2020; Manamgoda et al., 2014).

Traditionally, morphological characteristics have been used to identify *Bipolaris* species (Pham et al., 2015; Sun et al., 2020). However, researchers have been confused for years between three genera, *Cochliobolus*, *Bipolaris* and *Curvularia*, due to repeated name changes, refinements, and most crucially, similar or intermediate morphological traits between *Curvularia* and *Bipolaris* asexual phases (Manamgoda et al., 2012; Sivanesan, 1987). Concurrently, the taxonomy of the *Helminthosporium* species complex was also ambiguous. In the type species of graminicolous *Helminthosporium*, conidia are found at the tip of the geniculate conidiophore, and they continue to proliferate through sympodial extension from the subapical region (Alcorn, 1988), whereas conidia formed by *Helminthosporium velutinum* appear through small pores in the walls of distal and intercalary

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cells of conidiophores, and conidiophore growth stops with the development of terminal conidia (Goh et al., 1998; Luttrell, 1963). Based on these distinctive morphological difference, graminicolous *Helminthosporium* species were split into four genera, namely *Bipolaris*, *Curvularia*, *Drechslera* and *Exserohilum* (Luttrell, 1963; Shoemaker, 1959; Sivanesan, 1987). However, recently, molecular techniques have evolved to circumvent the challenges of conventional taxonomy in case of many complex groupings of plant pathogenic fungus. The species relationships in the genus *Bipolaris* can be better understood using a combination of morphological data and molecular taxonomy based on the ITS, GAPDH, TEF1α and LSU genes. (Berbee et al., 1999; Cai et al., 2011; Manamgoda et al., 2014; Udayanga et al., 2011).

Browntop millet (*Brachiaria ramosa*) is an important nutri-cereal that belongs to Poaceae family. The crop is gluten-free and rich in dietary nutrients, micronutrients, protein, fibre and vitamin B complex (Sarita, 2016). Due to its high nutritional value, low vulnerability to biotic stresses and extensive adaptation to climatic change, this crop is grown in India's marginal land, hills, tribal and rain-fed areas mostly by resource-poor farmers (Maitra, 2020).

Leaf blight symptoms on browntop millet (*Brachiaria ramosa*) were noticed for the first time during September 2018 in the All India Coordinated Research Projects (AICRP) on small millet experimental field (13.0784 N, 77.5793° E) at the University of Agricultural Sciences, GKVK, Bengaluru, India (Ramesh et al., 2021). Despite the fact that Misra and Prakash (1972) identified. *Helminthosporium setariae* as a plant pathogen in Bihar (North India), they did not present any scientific data or substantiate Koch's postulates. Furthermore, no cases of browntop millet leaf blight disease have been documented from the Indian subcontinent or anywhere else in the world. Therefore, the current study is the first to provide a comprehensive account of *Bipolaris* spp. on browntop millet, with the following objectives in mind: (1) Conduct a survey to determine the occurrence and severity of the disease; (2) Describe the causal agent using morphological and molecular techniques; and (3) Conduct laboratory tests to determine the pathogenicity and host range of the pathogen.

2 | MATERIALS AND METHODS

2.1 | Field survey and sampling

An intensive roving survey was conducted in 12 locations (65 fields) covering five major browntop millet growing states of India from September to November 2019 to assess the occurrence and distribution of leaf blight disease (Table 1). In each field, a W-shaped pattern was used to cover the entire area by making five stops, and ten plants were assessed for disease severity at each stop. The severity was determined visually using a 1–9 disease scale, with 1 =very slight infection, one or two small brown specks of pinhead size (0.1–1.0 mm), and less than 1% leaf area affected; 2 =light infection, brown lesions covering 1%–5% leaf area; 3 = 6%–10%, 4 =moderate infection covering 11%–20% leaf area, 5 = 21%–30%, 6 = 31%–40%, 7 =heavy infection, abundant spots covering 41%–50%, 8 = 51%–75% and many leaves dead; and 9 =very heavy infestation, lesions abundant on almost all leaves covering >75% leaf area, or plants dry prematurely (Figure 1), and the per cent disease index was calculated using Wheeler (1969).

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of leaves examined} \times \text{Maximum grade value}} \times 100$$

Representative leaf samples from each location exhibiting typical brown spot or leaf blight symptoms (Figure 2) were collected from tillering to maturing stage and transported to the laboratory for further analysis.

2.2 | Isolation and purification of fungal isolates

Fungal pathogen was isolated from the collected leaf samples on Potato Dextrose Agar (PDA) medium amended with streptomycin sulphate (500 ppm) to prevent the bacterial contamination (Kumar et al., 2011; Kusai et al., 2016). Isolates were purified on

TABLE 1 List of isolates, locality and severity of leaf blight disease on browntop millet

Sampling location (No. of fields surveyed)	District	State	Disease severity (%)	Designation of recovered isolates
Bengaluru (5)	Bengaluru	Karnataka	60.00	BTMH-1
Bengaluru (3)	Bengaluru Rural	Karnataka	55.00	-
V. C. Farm (6)	Mandya	Karnataka	18.00	BTMH-2
Arasikere (4)	Hassan	Karnataka	45.00	BTMH-3
Tipturu (5)	Tumakuru	Karnataka	43.00	BTMH-4
Chiknayakanhalli (4)	Tumakuru	Karnataka	39.50	-
Balajigapade (5)	Chikkaballapura	Karnataka	26.00	-
Berhampur (7)	Ganjam	Odisha	58.50	BTMH-5
Ranichauri (6)	Tehri Garhwal	Uttarakhand	39.75	BTMH-6
Nandyal (7)	Kurnool	Andhra Pradesh	51.00	BTMH-7
Gajularega (6)	Vizianagaram	Andhra Pradesh	42.10	BTMH-8
Kanke (7)	Ranchi	Jharkhand	37.50	BTMH-9

2% water agar medium using a modified single spore suspension method described by Chomnunti et al., (2011). In a test tube containing sterile water, a loop full of mycelial bit along with spore was placed and continuously stirred to release the spores. Using a pipette, the spore suspension was then evenly distributed on the surface of water agar media. After 12 hr of incubation at 26 ± 1°C, the plates were examined under a microscope to identify germinated conidia, and germinated spores were transferred to PDA plates individually (Choi et al., 1999).

2.3 | Morphology

For morphological studies, cultures were transferred to fresh Czapex Dox Agar medium after 10 days of incubation in the dark at 25°C. This study employed a total of nine isolates. Conidial masses from a 10-day-old culture were mounted in lactophenol and photographed with an Olympus BX 51 microscope and a Progres 2.7 version (Jenoptik, USA) digital camera. As parameters, macromorphological characteristics, such as colony colour, appearance, mycelial density and biomass, and micromorphological characteristics, such as conidial germination, size shape, conidiophore and hilum, were used.

FIGURE 1 Disease rating scale for the assessment of leaf blight disease severity on browntop millet leaves [Colour figure can be viewed at wileyonlinelibrary.com]

2.4 | DNA extraction, PCR amplification and phylogenetic analysis

The CTAB method, as described by Murray and Thompson (1980), was used to extract genomic DNA from all nine *Bipolaris* isolates, with minor modifications. The NanoDrop™ 1,000 Spectrophotometer (Thermo Fisher Scientific) was used to estimate the quality and quantity of genomic DNA. Following that, the DNA samples were diluted with nuclease-free water to a concentration of 100 ng/µl for PCR amplification. In a thermal cycler (Eppendorf Mastercycler V ProTM), gene fragments of ITS, GAPDH and LSU were amplified using primer pairs ITS1/ITS4 (White et al., 1990), gpd1/gpd2 (Berbee et al., 1999) and LR5/LROR (Schoch et al., 2009), respectively. PCR reactions were carried out in 25 µl reaction volume, contained 100 ng of DNA template, 2 mM of each dNTPs (Fermentas), 10 pmol of each forward and reverse primer, 25 mM $MgCl_2$ (Fermentas), 1X Taq buffer, 1 U *Taq* DNA polymerase (Fermentas) and nuclease-free water. The following were the PCR cycles: An initial 5 min denaturation at 94°C was followed by 35 cycles of denaturation for 45 s at 94°C, primer annealing for 45 s at 52°C and extension for 1 min at 72°C, followed by a final 10 min extension at 72°C. The amplified PCR products were separated by electrophoresis along with the 1kb DNA ladder (Bio Prep™ Cat No: BP010-R500) in 1.2% agarose gel stained with ethidium bromide (10 mg/ml). The gels were documented using a gel documentation system after electrophoresis (InGenius3 gel Doc-Syngene) Sequencing was outsourced to Chromous Biotech Pvt. Ltd., a Bengaluru-based private company.

The available sequences were then assembled and aligned using the Molecular Evolutionary Genetics Analysis (MEGA X) sequence alignment software. The aligned DNA sequences (ITS, LSU and GAPDH) of all the nine isolates were deposited in NCBI GenBank (http://www.ncbi.nlm.nih.gov). MEGA-X software was used to perform phylogenetic analysis for concatenated and individual genes, with 1,000 bootstrap repetitions (Kumar et al., 2018). The ITS, GAPDH and LSU gene reference sequences were obtained from NCBI GenBank and included in the tree. Table 2. lists the isolates from this study and the representative strains.

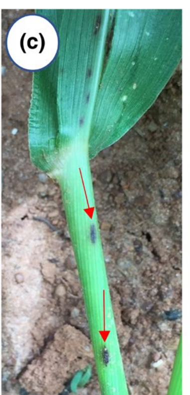

FIGURE 2 Disease symptoms on browntop millet caused by *B. setariae* (a) Small brown lesions with yellow halo on leaves. (b) Abundant lesions on almost all leaves. (c) Initial symptoms on leaf petiole. (d) Sever symptoms on plant stem [Colour figure can be viewed at wileyonlinelibrary.com]

2.5 | Pathogenicity and host range

Browntop millet (cv. Dundu korale) seedlings were raised in sterilized earthen pots with autoclaved sandy loam soil and fully decomposed vermicompost in a 2:1 volume ratio. For inoculation, a 10 ml conidial suspension was prepared separately from 10 days old pure cultures for each isolate (Table 1) by scraping pathogen colonies from the agar surface with a sterile spatula and sterile water, and the conidial solution was adjusted to 10^6 spores/ml. (Cipollone et al., 2020). Using an electric fine atomizer, the inoculum of corresponding isolates was sprayed equally on the foliage of 15-day-old browntop millet seedlings. The control plants, on the other hand, were only sprayed with

TABLE 2 Details of isolates used for the multi-gene DNA sequence analysis

Sl. No.	Organism	Strain/ Isolate	Location	GenBank accession numbers			Reference
				ITS	GAPDH	LSU	
1.	*Bipolaris bicolor*	CBS 690.96	-	KJ909762	KM042893	KM243287	Manamgoda et al., 2014
2.	*Bipolaris oryzae*	MFLUCC 100,715	Thailand	JX256416	JX276430	JX256384	Manamgoda et al., 2012
3.	*Bipolaris panici-miliacei*	CBS 199.29	Japan	KJ909773	KM042896	KM243281	Manamgoda et al., 2014
4.	*Bipolaris sorokiniana*	CBS 120.24	Italy	KJ909776	KM034821	KM243278	Manamgoda et al., 2014
5.	*Bipolaris victoriae*	CBS 327.64	USA	KJ909778	KM034811	KM243271	Manamgoda et al., 2014
6.	*Bipolaris secalis*	BRIP 14,453	Argentina	KJ415537	KJ415409	KJ415492	Tan et al., 2014
7.	*Bipolaris peregianensis*	BRIP 12,790	Zambia	JN601034	JN600977	JN601000	Manamgoda et al., 2014
8.	*Bipolaris gossypina*	BRIP 14,840	Kenya	KJ415528	KJ415418	KJ415481	Tan et al., 2014
9.	*Bipolaris eleusines*	CBS 274.91	Australia	KJ909768	KM034820	KM243289	Berbee et al., 1999
10.	*Bipolaris setariae*	CBS 141.31	USA	EF452444	EF513206	MH866609	Andrie et al., 2008
11.	*Exserohilum curvatum*	CBS 505.90	Venezuela	KT265252	LT715889	LT715620	Hernandez-Restrepo et al., 2018
12.	*Alternaria alternata*	CBS 965.95	India	KP124323	KP124178	KP124475	Woudenberg et al., 2015
13.	*Curvularia nodulosa.*	CBS 160.58	USA	JN601033	JN600975	JN600997	Manamgoda et al., 2012
14.	*C. brachyspora*	CBS 186.50	Java	KJ922372	KM061784	KM243268	Manamgoda et al., 2014
15.	*C. geniculata*	CBS 187.50	Indonesia	KJ909781	KM083609	KM243260	Manamgoda et al., 2014
16.	*C. gladioli*	ICMP 6,160	New Zealand	JX256426	JX276438	JX256393	Manamgoda et al., 2012
17.	*C. trifolii*	ICMP 6,149	New Zealand	KM230395	KM083607	KM243262	Manamgoda et al., 2014
18.	*C. tuberculata*	CBS 146.63	India	JX256433	JX276445	JX256401	Manamgoda et al., 2011
19.	*C. tripogonis*	BRIP 12,375	Australia	JN192388	JN600980	JN601002	Manamgoda et al., 2011
20.	*C. protuberata*	CBS 376.65	Scotland	KJ922376	KM083605	KM243264	Manamgoda et al., 2014
21.	*Bipolaris* spp.	BTMH-1	India	MT750299	MT896700	MT755709	This study
22.	*Bipolaris* spp.	BTMH-2	India	MT750300	MT896701	MT755710	This study
23.	*Bipolaris* spp.	BTMH-3	India	MT750301	MT896702	MT755711	This study
24.	*Bipolaris* spp.	BTMH-4	India	MT750302	MT896703	MT755712	This study
25.	*Bipolaris* spp.	BTMH-5	India	MT750303	MT896704	MT755713	This study
26.	*Bipolaris* spp.	BTMH-6	India	MT750304	MT896705	MT755714	This study
27.	*Bipolaris* spp.	BTMH-7	India	MT750298	MT896706	MT755715	This study
28.	*Bipolaris* spp.	BTMH-8	India	MT750297	MT896707	MT755716	This study
29.	*Bipolaris* spp.	BTMH-9	India	MT755708	MT896708	MT755717	This study

sterile water. Inoculated seedlings were maintained in the greenhouse at 25 ± 2°C and 80%–85% relative humidity with proper care. Observation on reaction of each isolate was recorded 2–12 days after postinoculation (DPI). Inoculation test was performed three times for each isolate, and the experiment was repeated twice. To prove Koch's postulates, the pathogen was re-isolated from symptomatic leaf tissues and morphological features were compared to the original isolates (Farag & Attia, 2020).

Host range was assessed by spraying the conidial suspension of virulent *Bipolaris* isolate (BTMH-5) followed by inoculation as described above on browntop millet and other small millets in the Poaceae family including Little millet (*Panicum sumatrense* Roth ex Roem. and Schult.), Barnyard millet (*Echinochloa frumentacea* Link), Finger millet (*Eleusine coracana* Gaertn.), Foxtail millet (*Setaria italica* (L.) P. Beauv.), Kodo millet (*Paspalum scrobiculatum* L.) and Proso millet (*Panicum miliaceum* L.).

3 | RESULTS

3.1 | Field survey

A total of 65 browntop millet fields in five major browntop millet growing states were inspected (Table 1). Bengaluru had the highest disease severity (60.00%), and Mandya had the lowest (18.00%) among the locations surveyed (Table 1). Most common symptoms include small brown pinhead size spots on leaves and stems, surrounded by yellow halo on both sides of leaves. As the disease

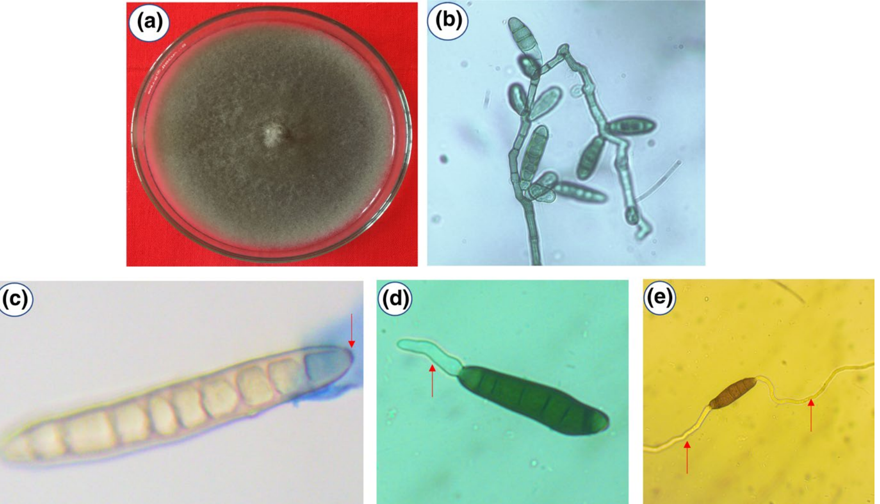

FIGURE 3 Morphological characteristics of *B. setariae*. (a) Colony appearance, (b) conidia on conidiophores, (c) multicelled conidia with protruded hilum, (d) unipolar and (e) bipolar conidial germination [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Cultural characteristics of *Bipolaris setariae* isolates on Czapek Dox Agar media

Isolate code	Days to cover full plate	Dates to full pigmentation	Colony colour	Surface and Topography	Margin	Mycelial density	Mycelium biomass (mg 100 ml^{-1})
BTMH-1	10	13	Greyish green	Flat	Irregular	Thin	430
BTMH-2	7	8	Olive green	Convex	Regular	Dense	370
BTMH-3	9	10	Dark grey or greyish black	Flat	Regular	Thin	360
BTMH-4	7	8	Greyish green	Flat	Regular	Thin	390
BTMH-5	6	7	Greyish green	Raised	Regular	Dense	470
BTMH-6	7	8	Olive green with white spots	Raised	Regular	Dense	420
BTMH-7	10	12	Greyish white	Umbonate	Regular	Dense at centre and thin at corners	450
BTMH-8	13	16	Dark grey or greyish black	Flat	Irregular	Thin	440
BTMH-9	13	15	Greenish white	Convex	Irregular	Dense	160

Note: All parameters are considered after observing three replicates per isolate.

TABLE 4 Summary of micromorphological data for *Bipolaris setariae* isolates

Isolates	Conidial shape	Conidial size (μm)		No. of septa		Hilar structure	Conidiophore width (μm)	Type of germination
		Range	Avg.	Range	Avg.			
BTMH-1	Fusoid, cylindrical to slightly curved	58.2–84 × 9.2–12.1	71.38 × 10.76	5–8	8	Slightly protruded	5.39	Bipolar and unipolar
BTMH-2	Fusoid, cylindrical to curved	69.3–120 × 10.8–14.6	101.06 × 12.89	6–9	9	Flat to slightly protruded	6.04	Bipolar and unipolar
BTMH-3	Cylindrical to slightly curved	38.5–85 × 10.3–17	70.55 × 14.3	5–9	8	Flat to slightly protruded	5.80	Bipolar and unipolar
BTMH-4	Cylindrical to curved	48.5–114.4 × 10.93–16.3	73.95 × 13.22	5–10	8	Flat to slightly protruded	5.32	Bipolar
BTMH-5	Subcylindrical to curved	65.17–104.63 × 9.62–11.27	81.41 × 10.91	6–9	9	Slightly protruded	5.92	Bipolar and unipolar
BTMH-6	Subcylindrical to curved	65.5–86 × 9.5–13.4	76.52 × 10.34	4–9	8	Flat to slightly protruded	6.04	Unipolar
BTMH-7	Fusoid, cylindrical to curved	70–130 × 9.8–13.1	87.65 × 11.35	6–10	8	Slightly protruded	5.49	Unipolar
BTMH-8	Subcylindrical to curved	49.4–65 × 8.3–12.6	59.46 × 10.69	4–8	6	Slightly protruded	4.92	Bipolar and unipolar
BTMH-9	Subcylindrical to slightly curved	62.3–89 × 10.5–13.8	76.28 × 11.69	4–9	7	Slightly protruded	5.57	Bipolar

Note: All parameters are considered after observing 30 conidia per isolate.

progress, the spots grow larger and coalesce, resulting in a blighted appearance (Figure 2).

3.2 | Isolation and morphological identification of fungal pathogen

Leaf blight infected samples collected during survey from different locations were subjected to pathogen isolation. *Bipolaris* isolates formed light grey to greyish white colonies with irregular margin and black colour pigmentation on reverse side of Petri plate (Figure 3a). A total of nine fungal pathogens isolates were recovered, and the isolates were given the designations BTMH-1 to 9 (Table 1). Mycelium was light brown to brown in colour, thin, septate and branched profusely. Conidiophores can be up to 120 μm long and 4.92–6.04 μm thick, dark brown in colour with intercalary and terminal conidia (Figure 3b). Conidia were fusiod, pale brown to dark brown, cylindrical with a slight curvature, with up to 8 pseudosepta and a slightly protruded hilum (Figure 3c). On the conidiophore, conidia were formed singly or in whorl. Seven days after inoculation, sporulation was seen. Conidia germinated in one of two ways: unipolar or bipolar (Figure 3d,e).

3.3 | Cultural and morphological characterization of Bipolaris setariae isolates

Czapek Dox Agar medium was used for cultural and morphological characterization of *B. setariae* isolates. In the medium, it took 6–13 days for isolates to develop 90 mm in diameter and 7–16 days for black pigmentation to appear. Slow growth was seen in isolates BTMH-8 and BTMH-9 (13 days), and fast growth was seen in isolate BTMH-5 (6 days). As indicated, the pigmentation of the incubation was described before, three isolates were greyish green (BTMH-1, BTMH-4 and BTMH-5), two isolates each were olive green (BTMH-2 and BTMH-6), dark grey (BTMH-3 and BTMH-8) and greyish white (BTMH- 7 and BTMH-9). On the medium, isolates produced flat, convex or elevated colonies. Four isolates (BTMH-1, BTMH-3, BTMH-4 and BTMH-8) showed poor and dense mycelial density and remaining five isolates had dense mycelium. Tables 3 and 4 offer a summary of the cultural and morphological data for all *Bipolaris* isolates.

3.4 | Molecular characterization

Clear bands of approximately 550 bp (ITS), 550 bp (GAPDH) and 900 bp (LSU) were obtained using those PCR primer pairs. The NCBI BLAST algorithm was used to confirm the identity of the generated sequences. Phylogenetic analysis of ITS sequences data showed maximum sequence similarity with two species namely *B. bicolara* and *B. setariae* but could not display any strong interspecies discrimination among the *Bipolaris* species studied. Similarly, sequence analysis of the LSU gene showed heterogeneous grouping of *Bipolaris* species. The GAPDH gene was found to be a better region for grouping the *Bipolaris* species, as it shared 99 per cent similarities with *Bipolaris setariae*, resulting in a single cluster (data not presented). However, maximum parsimony analysis of concatenated genes (ITS, GAPDH and LSU) revealed better interspecies differentiation than

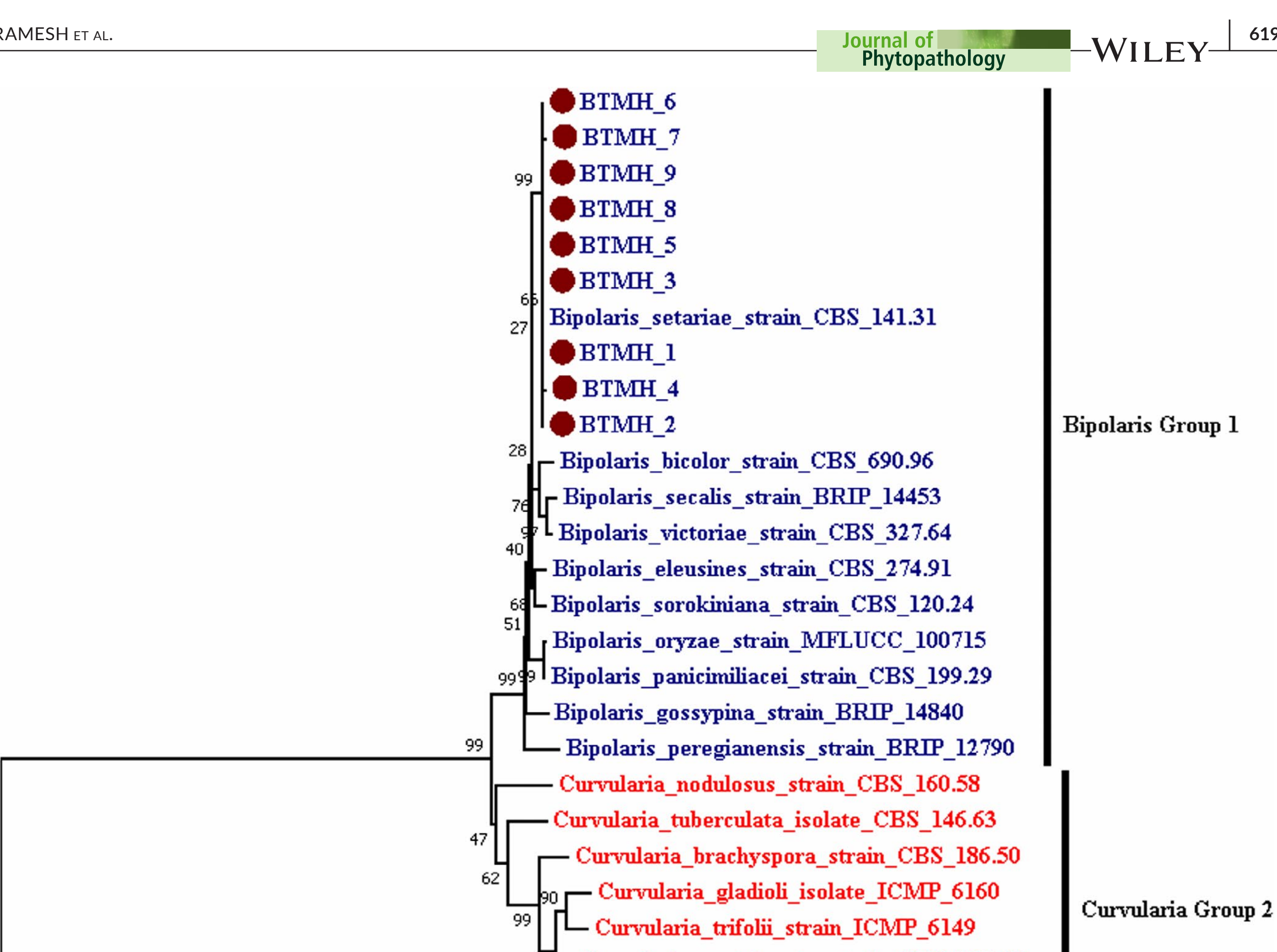

FIGURE 4 Multilocus phylogram generated by maximum parsimony analysis based on combined genes (ITS, GAPDH and LSU) sequences of *Bipolaris*, *Curvularia*, *Exserohilum* and *Alternaria* species [Colour figure can be viewed at wileyonlinelibrary.com]

any individual data set. All nine *Bipolaris* isolates were grouped with the type strain *Bipolaris setariae* and clustered into a single clade with clear resolution among the taxa studied, with all *Bipolaris* spp. clustered in group 1 and the sister genera *Curvularia* spp. clustered in group 2 (Figure 4).

3.5 | Pathogenicity and host range of B. setariae isolates

Pathogenicity tests demonstrated that under artificial inoculation conditions, all nine *B. setariae* isolates were pathogenic to browntop millet (cv. Dundu korale). Each of the nine isolates produced the same symptoms. Specifically, initially minute brown colour spots surrounded by a yellow halo appeared on the leaves; then, these minute brown spots grew in size and shape, resulting in a blighted appearance. Isolates BTMH-2, 3, 5 produced initial symptoms 2 DPI, while isolates BTMH-6, 7, 9 and BTMH-1, 4, 8 produced the symptoms 9 and 12 DPI, respectively (Figure 5). The pathogen was recovered from inoculated symptomatic plants and compared with original culture to satisfy Koch's postulates. In host range analysis, among the seven gramineous hosts tested, symptoms were developed only on browntop millet. However, the isolate BTMH-5 failed to infect other six small millets under artificial inoculation conditions.

4 | DISCUSSION

The study has observed and highlighted the presence of leaf blight disease in browntop millet (Figure 2). The symptoms were almost similar to leaf spot of maize caused by *B. setariae* (Xiao et al., 2019). Initial symptoms appeared on leaves as small brown

FIGURE 5 Pathogenicity test on browntop millet seedlings (captured on the 12th day after inoculation) performed by spraying spore suspension of nine *Bipolaris setariae* isolates. (a) BTMH-5; (b) BTMH-3; (c) BTMH-2; (d) BTMH-6; (e) BTMH-7; (f) BTMH-9; (g) BTMH-1; (h) BTMH-4 and (i) BTMH-8 [Colour figure can be viewed at wileyonlinelibrary.com]

to dark coloured spots or lesions of varied sizes and shapes, resulting in a blighted appearance. By using morphological and molecular characteristics, the pathogen was identified as *B. setariae* (Shoemaker), a member of the family Pleosporaceae as the causal agent of leaf blight disease in browntop millet, thus confirming new host report of this pathogen in India. For the first time, an extensive survey was done to determine the spread and severity of browntop millet leaf blight disease. The severity of the disease ranged from 18%–60% in the sites evaluated. Increased crop resilience due to agronomic interventions such as crop residue management and application of fungicide (mancozeb@ 2.5 gm/lt of water) at the time of disease emergence may have contributed to the minimum disease severity of 18% (Mandya) and 26% (Chikkaballapura). The current study's findings on disease severity are similar to those observed by Bhandari (2010). When compared to healthy plants, the incidence of wheat spot blotch (*B. sorokiniana*) disease is more severe under stress conditions and in weak plants. *Bipolaris* produced a brown-coloured, highly branched, septate mycelium. The conidia were multicelled, cylindrical with slightly curved having protruded hilum. The findings of this research are consistent with those of Alcorn (1988) and Manamgoda et al., (2012). Other distinguished microscopic characters such as conidial length, septation and conidial germination are in accordance with the earlier observations from Manamgoda et al., (2014) and Sun et al., (2020), although *B. setariae* shares similar morphological characters with *B. luttrellii*. It differs by producing concolorous conidia and few conidiogenous loci on conidiophore, while *B. luttrellii* mostly produces darker conidia and end cells with light colour (Manamgoda et al., 2014).

For species identification in *Bipolaris* and its sister genera, morphological data combined with molecular taxonomy based on the ITS, GAPDH, TEF1 and LSU genes have been recommended in recent years (Berbee et al., 1999; Manamgoda et al., 2014; Marin-Felix et al., 2017).

Concatenated phylogenetic analysis of ITS, GAPDH and LSU gene sequences showed better interspecies differentiation than any individual data set in the current study (Figure 4). Our findings are similar to those of Pham et al., (2015), Bhunjun et al., (2020) and Sun et al., (2020).

Members of the genus *Bipolaris* have been found to infect a wide range of hosts, including grass and non-grass species (Manamgoda et al., 2014; Tsukiboshi et al., 2005), of which only few pathogens of economic importance have been thoroughly investigated in terms of biology and host range (Kleczewski et al., 2012). *Bipolaris* species such as *B. sorokiniana*, *B. maydis* and *B. oryzae* have been found on multiple hosts. In contrast, several other species of *Bipolaris* including *B. clavata*, *B. microstegii* and *B. gossypina* have been reported to infect single host (Manamgoda et al., 2014). Despite the fact that this pathogen was first discovered in 1987 (Sivanesan), on *Echinochloa* spp., *Eleusine coracana*, *Eragrostia* spp., *Panicum* spp., *Pennisetum* spp. and *Setaria italica*, the results of the present study revealed that *B. setariae* did not infect *E. coracana*, *E. frumentacea*, *P. sumatrense*, *P. miliaceum* and *P. scrobiculatum*. This could be due to genomic changes in the pathogen genome over time, such as hybridization, horizontal gene transfer, point mutation, partial or complete gene deletion and nucleotide and/or amino acid substitution resulting in the host jump (Morris & Moury, 2019). For example, emergence of *Magnaporthe oryzae* on wheat was due to loss of function of a single avirulence gene (Inoue et al., 2017). *Blumeria graminis* f. spp. *triticale*, on the other hand, is a hybrid of two *B. graminis* subspecies that specialize in wheat and rye (Menardo et al., 2016). However, the genetic factor involved in the pathogen host jumps is largely unknown (Morris & Moury, 2019). *B. setariae* was extremely specific to browntop millet, according to the findings of this study. This could be owing to the above-mentioned mutations in the fungal genome making this pathogen specialized solely to browntop millet.

5 | FUTURE RESEARCH PROSPECTS TO CONTROL THE DISEASE

Leaf blight is a devastating disease that is threatening browntop millet production in India. High degree of variability in *Bipolaris* spp. remains a challenge for researchers, due to its ability to quickly overcome the host resistance. Although efforts are being made to exploit host resistance against *Bipolaris* in crops such as wheat, barley and maize, the resistance is mostly polygenic and

quantitative in nature. So far, no browntop millet cultivar has been identified that confers significant degree of resistance to the leaf blight disease. As a result, research into the discovery of novel resistance sources as well as the genetics of resistance need to be initiated. Unravelling host–pathogen relationship and development of durable resistant varieties through transgenic and CRISPR-Cas gene-editing technology should also become a priority. The pathogen, being hemibiotroph, survives on plant debris between the cropping seasons; these leftover crop residues are responsible for disease epidemics. So, it is important to reduce the fungal inoculum present in the soil. Therefore, one may also find suitable cultural practices, promising bioagents and effective chemical fungicides on an urgent basis to mitigate the yield losses caused by this disease. We believe that the information gathered in this study will be useful to plant pathologists and taxonomists in species identification and understanding the fungal epidemiology for better management of the disease.

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CONFLICT OF INTEREST

In connection to this article, the authors state that they have no conflicts of interest. All types of aid, including financial support, have been gratefully acknowledged.

AUTHOR CONTRIBUTIONS

K.B.P. and G.V.R. conceived the study. K.B.P., G.V.R., B.J. and H.D.V.K. designed the experiments. G.V.R., H.D.V.K. and A.K. performed the experiments. G.V.R., K.B.P., B.J., P.S.K., H.S.M. and H.D.V.K. analysed the data and interpreted the results. G.V.R., B.J., P.S.K. H.D.V.K and H.S.M. wrote the manuscript. K.B.P., T.E.N. and V.A.T. arranged funds, provided resources, reviewed the draft and provided critical scientific inputs for the improvement of the manuscript. All authors have read and approved the final manuscript.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The ITS, GPDH and LSU nucleotide sequence data that support the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/, and the remaining data sets generated for the present study are included within the article.

ORCID

Gutha Venkata Ramesh https://orcid.org/0000-0001-6197-523X
B. Jeevan https://orcid.org/0000-0002-8689-2497

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