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Confirmation of physiological race of *Bipolaris maydis* causing maydis leaf blight of maize in India

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ABSTRACT

Maydis leaf blight (MLB) incited by *Bipolaris maydis* occurs in most of the maize growing regions. Present study revealed morphological divergence among 74 isolates of *B. maydis* collected from geographically distant places of India. Based on morpho-cultural features clustered by R software, 25 representative isolates of *B. maydis* were used for race identification. The internal transcribed spacer (ITS) sequence further confirmed the isolates as *B. maydis*. In recent concept, variation in disease incidence and infectivity denotes genetic shift in the pathogen or introduction of new pathogenic race(s) through germplasm exchange. For unveiling Indian race(s) of *B. maydis*, present exploration was made. Four genetically divergent maize genotypes with cytoplasmic male sterility (CMS), viz. -C (MGU-161QPV-C), -T (MGU- 345PV-T), -S (MGU-150Q-S) and CM-119 (fertile cytoplasm) were assessed using 25 isolates of *B. maydis* during *kharif* 2018–19 at ICAR-IARI, New Delhi. The genotype MGU-161QPV-C associated with CMS-C was highly resistant followed by MGU-345PV-T (CMS-T) and MGU-150Q-S (CMS-S), whereas CM-119 showed high susceptibility. Present outcome confirmed prevalence of race 'O' of *B. maydis* in India and eliminated uncertainty about occurrence of other races, i.e. T and C.

Keywords: *Bipolaris maydis*, Maize, Maydis leaf blight, Race

Maize is the third most worthwhile cereal crop in India after rice and wheat. Globally maize is cultivated in 193.7 mha beyond 165 countries, with an overall production and average productivity of 1147.6 mt, 5.92 t/ha, respectively (FAOSTAT 2018). Maize production is plagued by multifarious stresses leading to significant yield losses. Maydis leaf blight (MLB), banded leaf and sheath blight, downy mildews, rust, smut, and post flowering stalk rots are of prime importance and inflict about 13% losses in quantity and quality of grains (Yadav *et al.* 2015). Of them, MLB incited by *Bipolaris maydis* (Nishik. & Miyake) Shoemaker [teleomorph: *Cochliobolus heterostrophus* (Drech.) Drech.] reported from various parts of India, is a prime menace of maize production. Smith *et al*. (1970) discriminated two races (T and O) of *C. heterostrophus* based on differential pathogenic reactions on maize inbreds. MLB, mainly triggered by race O, is a peril to sweet corn in southern Atlantic coast region of the USA. It can be responsible for yield reductions of 40% or more (Byrnes *et al*. 1989). Payak and Sharma (1978) documented 30.3% yield loss in susceptible maize cultivars infected by the *B. maydis* race O. Alcorn and Pont (1973) reported *C. heterostrophus* race T from several graminaceous plants in Queensland. Both, race T and O distributed globally, whereas the race C confined only to China (Wei *et al*. 1988).

Tagtmeier *et al*. (1982) confirmed that race O is extremely virulent to maize lines associated with fertile/ normal cytoplasm. The pathogenic races distinguished in their response to cytoplasmic male sterility (CMS) of the host. Wei *et al.* (1988) noticed race C of *B. maydis* in China. Thus, three races of *H. maydis* were reported so far, namely race T, O and C. But in India, information is scanty regarding presence of various races of *B. maydis*. This erratic prevalence of MLB in India provoked us to study on racial differences in *B. maydis* and resolve the doubt on race type. The resultant information will be useful in its management strategies especially in developing resistant cultivars against the aggressive race of *B. maydis*.

MATERIALS AND METHODS

Collection and isolation of the pathogen: Seventy four MLB disease samples were collected from geographically distinct sites of thirteen states of India in *kharif* 2017 (Table 1). The incitant was isolated and purified using standard protocol. The isolated pathogen was identified based

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on colony and spore characteristics as per the standard description of the species given in CMI Descriptions of Pathogenic Fungi and Bacteria (1952).

Morpho-cultural characterization: Morpho-disparity in the isolates of *B. maydis* was assessed by analyzing discrete cultural and phenotypic attributes like size of conidia and septa number. Cultural characteristics of the fungus were studied on potato dextrose agar (PDA) following standard methodologies (Sivanesan 1987, Karimi 2003). The tentatively identified 74 isolates were placed into different clusters based on morphological characters using R software version 3.4.3. Of these, 25 representative isolates were used for further studies.

Molecular identification of the pathogen

DNA extraction and PCR amplification of ITS regions: DNA was isolated from 25 isolates of *B. maydis* by CTAB (cetyl trimethyl ammonium bromide) method as described by Murray and Thompson (1980). PCR amplification of ITS region of 25 representative isolates was done using ITS1 (Forward primer, 5' TCCGTAGGTGAACCTGCGG 3′) and ITS4 (Reverse primer, 5' TCCTCCGCTTATTGATATGC 3') (White *et al.* 1990). The amplified products of 596 bp of all isolates were sequenced. The trimmed sequences subjected to nucleotide Blast in NCBI website to identify the organism. ITS sequences of 25 isolates were submitted to NCBI database and accession numbers of the sequenced isolates were obtained.

Confirmation of physiological race of the pathogen

Host and pathogen inoculation: In order to evaluate reaction of genetically different background genotypes to *B. maydis*, three cytoplasmic male sterile lines, viz. CMS S (HGO-MSS-150Q), CMS T (HGO-MST-V345PV), CMS C (HGO-MSC-161QtPV) and one normal cytoplasmic line CM 119 (fertile cytoplasm) were obtained from Maize Breeding Lab of the Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), New Delhi. The seeds of CMS lines were sown during *kharif* (June to October) 2018–19 in maize field of the Division of Plant Pathology, IARI, New Delhi (GPS: latitude of 28.04° N and longitude of 77.12° E). Field experiment was laid out with 25 plots. Each plot contained four rows (Fig 1). The width between two rows was 75 cm, whereas length of each row was 2 m. To avoid cross infection of the isolates of *B. maydis*, 20 m distance between the two plots was maintained by raising the barrier rows of maize plants. In each spot four genotypes CMS S (HGO-MSS-150Q), CMS T (HGO-MST-V345PV), CMS C (HGO-MSC-161QtPV) and one normal cytoplasmic line CM 119 (fertile cytoplasm) were sown. Standard package of practices of maize cultivation was followed.

Mass multiplication of the pathogen was done on sorghum seeds using pure culture of each isolate of *B. maydis* (Payak and Sharma 1983). Apart from natural disease pressure, plants were inoculated to create artificial epiphytotics. First inoculation was done by showering optimum quantity of inoculum powder into the central leaf whorl of 25 days old plants at 3-4 leaf stage (Payak and Sharma 1983). Second inoculation was done on 35 days old plants to develop high disease pressure.

Fig 1 General view of the field layout depicting various grades of maydis leaf blight disease reaction in four cytoplasmically different maize genotypes. Note severe infection in the genotype CM 119 with normal cytoplasm caused by one isolate of *Bipolaris maydis* race O. The genotype CMS C was free from MLB symptoms while CMS T and CMS S had mild infection of the same race of *B. maydis.*

Disease assessment: Disease reaction was scored 20-25 days after second inoculation at maximum level of infection by using 1-9 scale (Mitiku *et al.* 2014).

RESULTS AND DISCUSSION

Maydis leaf blight caused by *B. maydis* is a serious threat to the maize production globally as well as in India**.** Seventy four isolates collected from geographically different sites were investigated for morphological and cultural traits. There was variation in growth pattern, color, texture of the colony, behavior, sporulation rate, conidia size, length, width of conidia of 25 representative isolates (Table 1). Such prominent disparity was observed among the isolates of different sites as well as among the isolates of the same region which might be ascribed to their inherent physiological and genetic characters. For this nature, few isolates showed ability in adaptation to the particular environment. Previously, Ishar Pal *et al*. (2015) noticed large sized conidia in the MDCh1 (Delhi) isolate of *B. maydis* while smallest was identified in MUCh3 (Udaipur) isolate.

Out of 74 isolates of *B. maydis*, 25 isolates were selected based on clusters (Fig 2) made by R statistical software version 3.4.3 using different morpho-cultural traits. These 25 representative isolates were subjected to PCR amplification of ITS region and it resulted in amplification of single band at 596 bp. Subsequently PCR products were sequenced. The BLAST analysis of 25 ITS sequences in NCBI database exhibited 99-100% similarity with *B. maydis* (Table 1). The trimmed and curated ITS sequences of 25 isolates were deposited in the NCBI GenBank and obtained accession numbers. Sun *et al*. (2020) collected isolates causing leaf spot of maize in China and confirmed as *B. maydis* based on ITS and gpdh gene sequences. In Indian context, Gogoi *et al*. (2014) also identified *B. maydis* based

Table 1 Isolates of *Bipolaris maydis* collected from different maize growing regions of India during *kharif* 2017, their morpho-cultural features and *GenBank* accession numbers

Assigned code of isolate*	Genus/ species identity	Place	State	Morpho-cultural features						ITS sequence	
				Length of conidia (µm)	Width of conidia (µm)	No. of septa	Colony growth (cm)	Colony color-surface texture	Colony margin shape	Sequence length (bp)	GenBank Accession Number
Bm1_ Almora1	*B. maydis*	Almora	Uttarakhand	58.53	13.28	4.0	5.0	Brown-rough	Irregular	511	MT150649
Bm2_ Anand1	*B. maydis*	Anand	Gujarat	46.94	11.09	3.0	4.0	Brown-smooth	Irregular	541	MT150650
Bm3_ Bangalore1	*B. maydis*	Bangalore	Karnataka	35.04	8.39	3.0	6.8	Brown-smooth	Irregular	511	MT150651
Bm4_ BariBrahmna1	*B. maydis*	Bari Brahmna	Jammu and Kashmir	46.25	14.25	5.0	4.6	Brownish white-smooth	Irregular	512	MT150652
Bm5_ Bhubaneswar1	*B. maydis*	Bhubaneswar	Odisha	46.67	10.79	5.0	4.2	Black-rough	Irregular	512	MT150653
Bm6_ Dharwad1	*B. maydis*	Dharwad	Karnataka	65.16	12.93	8.0	4.8	Black-smooth	Regular	541	MT150654
Bm7_ Haldwani1	*B. maydis*	Haldwani	Uttarakhand	72.53	13.09	7.0	4.0	Blackish white-smooth	Irregular	540	MT150655
Bm8_ Hyderabad1	*B. maydis*	Hyderabad	Telangana	76.58	13.2	8.0	5.0	Black-smooth	Regular	540	MT150656
Bm9_ IARI1	*B. maydis*	IARI, Delhi	Delhi	50.95	11.31	4.0	4.0	Black-rough	Regular	540	MT150657
Bm10_ IARI2	*B. maydis*	IARI, Delhi	Delhi	48.65	12.97	3.0	3.0	White-rough	Regular	543	MT150658
Bm11_ IARI3	*B. maydis*	IARI, Delhi	Delhi	62.64	12.43	8.0	2.5	White-smooth	Regular	527	MT150659
Bm12_ Kangra1	*B. maydis*	Kangra	Himachal Pradesh	61.1	14.67	6.0	5.5	Black-rough	Regular	541	MT150660
Bm13_ Karnal1	*B. maydis*	Karnal	Haryana	54.49	14.84	4.0	4.5	Brownish white-smooth	Irregular	544	MT150661
Bm14_ Kinnaur1	*B. maydis*	Kinnaur	Himachal Pradesh	50.07	11.68	5.0	3.0	White-smooth	Regular	530	MT150662
Bm15_ Kullu1	*B. maydis*	Kullu	Himachal Pradesh	71.52	15.69	7.0	3.1	Brownish white-rough	Regular	540	MT150663
Bm16_ Ludhiana1	*B. maydis*	Ludhiana	Punjab	56.97	15.21	5.0	3.5	Brownish white-smooth	Irregular	527	MT150664
Bm17_ Mandi1	*B. maydis*	Mandi	Himachal Pradesh	56.99	13.55	5.0	3.0	Brown-rough	Regular	509	MT150665
Bm18_ Mau1	*B. maydis*	Mau	Uttar Pradesh	66.02	14.16	6.0	5.9	Brownish white-smooth	Regular	515	MT150666
Bm19_ Motipur1	*B. maydis*	Motipur	Bihar	50.57	13.76	4.0	3.5	White-smooth	Regular	518	MT150667
Bm20_ Palampur1	*B. maydis*	Palampur	Himachal Pradesh	43.7	10.49	3.0	3.7	Black-rough	Regular	527	MT150668
Bm21_ Pantnagar1	*B. maydis*	Pantnagar	Uttarakhand	47.57	10.25	3.0	5.8	Brown-rough	Regular	543	MT150669
Bm22_ RakhDhiansar1	*B. maydis*	RakhDhiansar	Jammu and Kashmir	86.01	16.01	8.0	4.8	White-smooth	Regular	542	MT150670
Bm23_ Ranchi1	*B. maydis*	Ranchi	Jharkhand	92.16	14.64	8.0	2.8	Brown-smooth	Regular	541	MT150671
Bm24_ Samastipur1	*B. maydis*	Samastipur	Bihar	48.36	11.18	5.0	3.7	Brown-rough	Regular	541	MT150672
Bm25_ Solan1	*B. maydis*	Solan	Himachal Pradesh	68.06	12.94	7.0	2.5	Black-rough	Regular	540	MT150673

*Representative isolates of *B. maydis* out of 74 isolates used for detail study

Table 2 Disease reaction pattern incited by 25 representative isolates of *Bipolaris maydis* on four maize genotypes with different cytoplasmic background

Isolate	Score of MLB on maize genotypes (1- 9 scale)															
	CM 119				CMS S (HGO-MSS-150Q)				CMS T (HGO-MST-V345PV)				CMS C (HGO-MSC-161QtPV)			
	2018	2019	Pooled	Reaction	2018	2019	Pooled	Reaction	2018	2019	Pooled	Reaction	2018	2019	Pooled	Reaction
Bm1_Almora1	8.0	8.0	8.0	S	4.0	5.0	4.5	MR	3.0	4.0	3.5	MR	1.0	2.0	1.5	R
Bm2_Anand1	7.0	6.0	6.5	MS	2.0	3.0	2.5	R	4.0	2.0	3.0	R	1.0	1.0	1.0	R
Bm3_Bangalore1	7.0	8.0	7.5	S	3.0	3.0	3.0	R	2.0	3.0	2.5	R	1.0	1.0	1.0	R
Bm4_Bari Brahmna1	7.0	8.0	7.5	S	4.0	3.0	3.5	MR	3.0	4.0	3.5	MR	1.0	2.0	1.5	R
Bm5_Bhubaneswar1	8.0	8.0	8.0	S	3.0	4.0	3.5	MR	2.0	3.0	2.5	R	1.0	2.0	1.5	R
Bm6_Dharwad1	5.0	9.0	7.0	MS	3.0	3.0	3.0	R	2.0	2.0	2.0	R	1.0	1.0	1.0	R
Bm7_Haldwani1	7.0	7.0	7.0	MS	4.0	3.0	3.5	MR	2.0	3.0	2.5	R	1.0	2.0	1.5	R
Bm8_Hyderabad1	7.0	8.0	7.5	S	1.0	4.0	2.5	R	1.0	3.0	2.0	R	1.0	1.0	1.0	R
Bm9_Delhi 1	8.0	9.0	8.5	S	5.0	5.0	5.0	MR	3.0	4.0	3.5	MR	2.0	3.0	2.5	R
Bm10_Delhi 2	6.0	7.0	6.5	MS	1.0	2.0	1.5	R	2.0	2.0	2.0	R	1.0	1.0	1.0	R
Bm11_Delhi 3	7.0	6.0	6.5	MS	4.0	5.0	4.5	MR	3.0	4.0	3.5	MR	1.0	2.0	1.5	R
Bm12_Kanga1	5.0	8.0	6.5	MS	2.0	3.0	2.5	R	2.0	2.0	2.0	R	1.0	1.0	1.0	R
Bm13_Karnal1	8.0	8.0	8.0	S	4.0	5.0	4.5	MR	2.0	2.0	2.0	R	1.0	1.0	1.0	R
Bm14_Kinnaur1	6.0	5.0	5.5	MS	2.0	1.0	1.5	R	2.0	1.0	1.5	R	1.0	1.0	1.0	R
Bm15_Kullu1	8.0	7.0	7.5	S	4.0	5.0	4.5	MR	3.0	4.0	3.5	MR	1.0	2.0	1.5	R
Bm16_Ludhiana1	6.0	6.0	6.0	MS	4.0	5.0	4.5	MR	1.0	5.0	3.0	R	1.0	1.0	1.0	R
Bm17_Mandi1	7.0	4.0	5.5	MS	2.0	2.0	2.0	R	2.0	1.0	1.5	R	1.0	1.0	1.0	R
Bm18_Mau1	7.0	8.0	7.5	S	3.0	3.0	3.0	R	2.0	3.0	2.5	R	1.0	1.0	1.0	R
Bm19_Motipur1	6.0	5.0	5.5	MS	1.0	2.0	1.5	R	2.0	2.0	2.0	R	1.0	1.0	1.0	R
Bm20_Palampur1	6.0	9.0	7.5	S	2.0	5.0	3.5	MR	1.0	4.0	2.5	R	1.0	1.0	1.0	R
Bm21_Pantnagar1	7.0	9.0	8.0	S	3.0	4.0	3.5	MR	3.0	5.0	4.0	MR	1.0	2.0	1.5	R
Bm22_Rakh Dhiansar1	5.0	8.0	6.5	MS	2.0	3.0	2.5	R	2.0	3.0	2.5	R	1.0	1.0	1.0	R
Bm23_Ranchi1	7.0	8.0	7.5	S	2.0	5.0	3.5	MR	1.0	4.0	2.5	R	1.0	1.0	1.0	R
Bm24_Samastipur1	6.0	7.0	6.5	MS	2.0	5.0	3.5	MR	1.0	3.0	2.0	R	1.0	1.0	1.0	R
Bm25_Solan1	6.0	8.0	7.0	S	4.0	2.0	3.0	R	1.0	2.0	1.5	R	1.0	1.0	1.0	R

R, Resistant; MR, Moderately resistant; S, Susceptible; MS, Moderately susceptible.

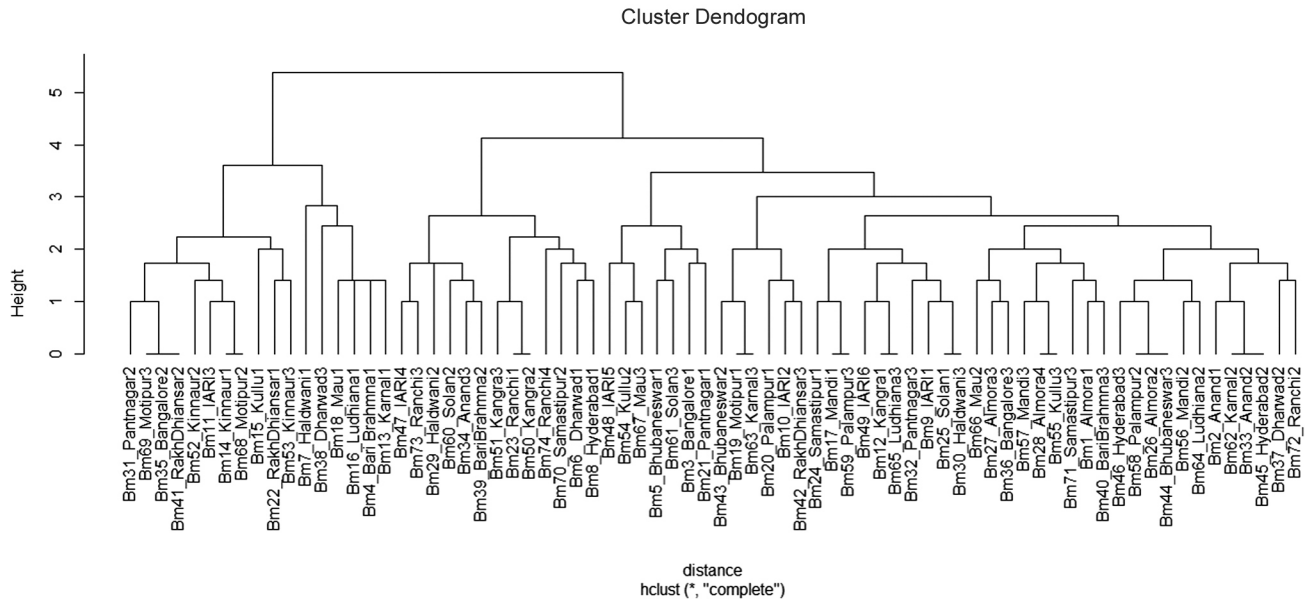

Fig 2 Morpho-cultural characters based dendogram of 25 isolates of *Bipolaris maydis*.

on ITS sequences and ITS-RFLPs.

Further, 25 representative isolates of *B. maydis* were used for their race identification. Their infectivity on maize host was investigated by inoculating three different types of male sterile cytoplasmic genotypes, *viz.* CMS T (HGO-MST-V345PV), CMS S (HGO-MSS-150Q), CMS C (HGO-MSC-161QtPV) and one normal cytoplasmic composite maize line CM 119 (Table 2). The CM 119 genotype was highly susceptible to all tested isolates of *B. maydis* during both *kharif* 2018 and 2019 seasons (Fig 1). In contrast, CMS S (HGO-MSS-150Q) was moderately resistant to all the isolates. CMS T (HGO-MST-V345PV) showed resistant reaction, whereas CMS C (HGO-MSC-161QtPV) exhibited highly resistant reaction to the isolates of *B. maydis* on which very minute lesions were developed. It was noted that enlargement of MLB lesions continued till maturity of the crop on CM119 only for which the leaves were completely blighted (Fig 1). However, none of the genotypes showed wilting symptoms due to infection of the tested fungal isolates. The characteristics of the race T of *B. maydis* is the ability to cause infection of cob husks apart from the leaves (Agrios 2005). But in our study, no any visible symptoms of MLB were observed on the cobs of CMS T line. In India race T was reported elsewhere, but race 'O' was documented as the most prevailing one (Sharma and Rai 2005). Moreover, there has not yet been made any conclusive proof on the existence of race T of *B. maydis* in India. Tagtmeier *et al*. (1982) reported that the race O is extremely virulent on the corn lines of normal cytoplasm (N-c). In China, Wei *et al*. (1988) observed *B. maydis* isolates collected from infected maize leaves produced comparatively larger lesions on the leaves of CMS C than those corn lines possessing CMS T, CMS S, or normal (N) cytoplasm. Hence the isolates showing virulence specifically to the CMS C corn were designated as race C, which is a unique race of *B. maydis* existing in China only. Earlier study in Japan conducted by Tskiboshi *et al*. (1996) revealed that all *B. maydis* isolates collected from symptomatic maize leaves were highly pathogenic on normal cytoplasmic maize lines than in the CMS T and CMS C lines and designated all the Japanese isolates of *B. maydis* as the race O. Similarly results of our investigation also clearly indicated about predominance of only the race O of *B. maydis* in India. The pathogenic race identification and ascertaining its prevalence are important pre-requisites for development of resistant varieties of any crop in the disease prone regions.

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