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STUDIES ON THE CULTURAL CHARACTERS AND PATHOGENICITY STUDIES OF BROWN LEAF SPOT OF RICE CAUSED BY *HELMINTHOSPORIUM ORYZAE* (SYN: *BIPOLARIS ORYZAE*)

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Abstract

The paper deals with the identifying the cultural characters of Brown leaf spot of rice caused by *Helminthosporium oryzae* ((Syn: *Bipolaris oryzae*) pathogen and identifying the most virulent isolate. Five isolates are collected from various places and identified isolates of *H.oryzae* were designated as Ho_1 (Puthoor), Ho_2 (Kathiramangalam), Ho_3 (Annamalainagar), Ho_4 (Keerapalayam) and Ho_5 (Kodipallam). All the five isolates of *H. oryzae* (Ho_1, Ho_2, Ho_3, Ho_4 and Ho_5) exhibited difference in the morphological characters such as colony characters, length and width of the conidium, colour of the conidia etc., Among the five isolates of *H.oryzae*, the isolate (Ho_1) collected from Puthoor village was found to be more virulent recording maximum brown spot incidence. The isolate Ho_5 collected from Kodipallam village was the least virulent.

Key words **:** Spore colony characters, Pathogenicity studies, Brown leaf spot of rice

Introduction

Rice (*Oryza sativa* L.) is the second most cultivated crop worldwide and it has been estimated that half the world's population survives wholly or partially on this crop (Van Nguyen and Ferrero, 2006) and rice provides more calories per ha than any other cereal food grains. Also, rice crop has been under cultivation from time immemorial, being grown under varying climatic conditions in different parts of the world. Rice crop is widely affected by a number of diseases caused by fungi, bacteria, viruses and mycoplasma which results in considerable yield losses (Ou, 1985). Among the various fungal diseases of rice, brown spot or sesame leaf spot incited by *Helminthosporium oryzae* (Breda de Haan) Subram. and Jain (Syn: *Bipolaris oryzae* (Breda de Haan) Shoemaker) is found to occur in most rice growing areas. The disease is also referred as fungal blight or Helminthosporiose. In India, the first occurrence of the disease was perhaps during 1918-1919 in the deltaic tracts of Godavari and Krishna (Sundaraman, 1922). The major objectives of this paper are identifying the cultural characters of pathogen and identifying the most virulent isolate.

Materials and Methods

Isolation and maintenance of culture (*in vitro*)

The pathogen *H. oryzae* was isolated from brown spot infected leaves of rice collected from the villages which showed the maximum brown spot disease incidence in each region by the tissue segment method (Rangaswami, 1972). The affected leaf portions were cut into small bits, surface sterilized with 0.1 per cent mercuric chloride solution for one min., washed using sterile dist. water and then placed on special medium *viz*., Rice Polish Agar Medium (Gangopadhyay, 1984) and incubated at room temp. (28 ± 3°C) for seven days. The fungus was subsequently purified by single spore isolation and the culture was maintained both on Czapek's (Dox) Agar and Potato Dextrose Agar slants for further studies. Slants were examined periodically for purity, viability and pathogenicity of *H. oryzae*.

Cultural Characteristics of the Isolates

Fifteen ml of the sterilized PDA medium was poured into sterile Petri dishes and allowed to solidify. A nine mm culture disc from each isolate of *H. oryzae* was aseptically placed at the centre of the dish and incubated at room temperature for seven days under room temperature (28±2°C). After incubation the mycelial growth, colour and spore characters were assessed and recorded. Micrometric measurements of conidia was done after staining with lactophenol cotton blue and observed under microscope. The identified isolates of *H.oryzae* were designated as Ho_1 (Puthoor), Ho_2 (Kathiramangalam), Ho_3 (Annamalainagar), Ho_4 (Keerapalayam) and Ho_5 (Kodipallam).

Pathogenicity Studies

The pathogenicity of the fungus was tested on ADT 36 rice variety raised for this purpose in pots.

Spore suspensions with adequate cfu *viz*., 50,000 spores/ml were prepared in two per cent sucrose solution and the plants were sprayed with an atomizer. The inoculated seedlings/pots were maintained in polyhouse with frequent sprinkling of water to maintain high humidity to favor conditions for infection. Development of characteristic symptoms of brown spot was assessed and the pathogen was reisolated from the infected leaves and compared with the original culture in special medium. The isolate collected from Annamalai nagar proved as the most virulent isolate as it registered the maximum Per cent Disease Incidence in the pathogenecity studies.

Results and Discussion

Morphological Characters of *H. Oryzae*

In the present study, from each region the villages which showed the maximum disease incidence were chosen for the isolation of the pathogen. Thus, five isolates of *Helminthosporium* sp. were isolated, identified and designated as Ho_1, Ho_2, Ho_3, Ho_4 and Ho_5 (Table 2).

The colony characters of the isolates (Ho_1, Ho_2, Ho_3, Ho_4 and Ho_5) were observed visually and results are presented in table 1. Colony morphology of all the isolates showed olivaceous, light brown to black, septate, profuse aerial/submerged and branched mycelium. The colour of the conidia was brown to light brown colour. The conidia were slightly curved with a bulge in the middle and tapering towards the ends and when fully mature they were brownish in colour.

The size of the conidia significantly varied among the isolates from 29.3 to 33.2 µm length and 13.5 to 14.8 µm width. The maximum conidial length was observed in Ho_1 followed by Ho_3, Ho_5, Ho_4 and Ho_2 in the decreasing order of merit. The maximum conidial width was observed in Ho_5 followed by Ho_4, Ho_1, Ho_2 and Ho_3 in the decreasing order of merit. The number of septations ranged from 3 to 5 and maximum was observed in Ho_4.

The colony morphology of all the isolates showed olivaceous, light brown to black, septate, profuse aerial/submerged and branched mycelium. The colour of the conidia was brown to light brown colour. Sakamoto (1934) noticed that *H. oryzae* had branched mycelium and septate conidia with variations in size. A similar observation was made in the present study with reference to the mycelium and conidia of Ho_3 isolate. The isolates of *H. oryzae* causing brown spot of rice were divided into several groups based on morphological and cultural characters (Vijayakumar, 1998).

In the present study, the size of the conidia significantly varied among the isolates from 29.3 to 33.2 µm length and 13.5 to 14.8 µm width with septations ranging from 3 to 5. Similar to the present observations the variation in length and breath with individual isolates was reported by earlier investigators (Unnikrishna Pillai, 1988; Vidhyasekaran *et al.,* 1991; Prasad and Bharat, 1995; Harish *et al.,* 2007). These earlier reports lend support to the present findings.

Pathogenecity of *H. oryzae* Native Isolates

The data depicted in table 2 revealed varied levels of pathogenecity with different isolates. Among the five isolates of *H. oryzae* collected from different conventionally rice growing areas, the isolate Ho_1 collected from Puthoor village of Kollidam region was found to be more virulent recording maximum disease incidence of 67.56 per cent (75DAT) followed by Ho_3 (58.20 %) collected from Annamalainagar village. The isolates Ho_2 and Ho_5 showed 44.00 and 43.92 per cent disease incidence and they were on par with either other. The isolates Ho_1 and Ho_3 recorded 43.50 and 38.85 per cent disease incidence at 45 DAT itself. The most virulent isolate of *H.oryzae* (Ho_1- Puthoor) was identified in this study.

The variability in the pathogenicity among the isolates of *H.oryzae* was reported by several workers (Lee *et al.,* 1984; Prasad and Bharat, 1995; Harish *et al.,* 2007; Peeyush Kumar *et al.,* 2011). The variations in brown spot incidence in different locations could well be attributed to the difference in virulence of the *H. oryzae* isolates. Harish *et al.* (2007) mentioned that among the fifteen isolates collected from various areas of Tamil Nadu, the isolate collected from Ammapettai, Thanjavur district recorded maximum (82.96 %) brown spot disease index in ADT 36 variety. Vijayakumar, (1998) also mentioned that variability in virulence among the five isolates *H. oryzae* on rice. In the present study, the disease incidence was the maximum in 75 DAT plants. A similar observation was made by Misra (1973) with regard to *H. oryzae* infection in rice. The susceptibility of rice plant to the disease at the later stages of growth might be due to the decline in the *in situ* defense mechanism. The above reports are in agreement with the present investigation.

Table 1 : Morphological characters of *H. oryzae* native isolates of selected villages

Characters	Puthoor Ho_1	Kathiramangalam Ho_2	Annamalainagar Ho_3	Keerapalayam Ho_4	Kodipallam Ho_5
Colony characters	Olivaceous, profuse aerial / submerged mycelium with fine texture, with many branches	Light brown, profuse aerial mycelium, septate branched	Olivaceous, pale olive in maturity medium fine and profuse, septate.	Olivaceous, profuse aerial mycelium with medium, fine texture, septate branches	Black velvety mycelial mat, profuse fine mycelium and completely oppressed, septate, simple
Conidia – Length (µm)	33.2	29.3	31.8	30.5	31.0
Conidia – Width (µm)	14.0	13.8	13.5	14.2	14.8
Colour of the conidium	Brown	Light brown	Light brown	Brown	Brown
No. of septations per conidium	3	4	4	5	3

Table 2 : Pathogenecity of *H. oryzae* native isolates

Isolates	Brown spot incidence					Mean
	25 DAT	30 DAT	45 DAT	60 DAT	75 DAT	
Puthoor Ho_1	15.25	27.80	43.50	51.65	67.56	41.15
Kathiramangalam Ho_2	12.44	19.25	27.59	36.00	44.00	27.85
Annamalainagar Ho_3	13.04	22.64	38.85	47.43	58.20	36.03
Keerapalayam Ho_4	12.80	20.45	30.01	39.12	49.68	30.41
Kodipallam Ho_5	10.68	17.83	23.16	31.54	43.92	25.42
C.D. (p=0.05)	1.46	1.93	1.85	1.39	2.10	

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