



MORPHOLOGICAL CHARACTERIZATION OF *BIPOLARIS ORYZAE* CAUSING BROWN SPOT OF PADDY IN BIHAR

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ABSTRACT

An extensive survey was conducted during kharif season of 2012 and 2013 in major rice growing areas of Bihar for observing disease incidence of brown spot of paddy and for collection of diseased samples of paddy (leaf as well as seed) to study morphological variability amongst the isolates of Brown spot fungus, *Bipolaris oryzae*. These samples from 25 districts were used for isolation of desired pathogen from spotted leaf and seeds with infection of brown spot disease. The disease was prevalent at all the locations surveyed. 52 isolates of *Bipolaris oryzae* from rice were characterized morphologically. On the basis of colony morphology and growth pattern on PDA, these all isolates can be grouped into 5 categories: Black with fluffy growth (16 isolates), Black with suppressed growth (10 isolates), Grey with cottony growth (9 isolates), Grey and white mix with cottony growth (12 isolates) and white with cottony growth (5 isolates). Based on colony diameter all these isolates can be grouped into 3 different categories: Slow growing 9 isolates), Moderate growing (15 isolates) and Fast growing (28 isolates). Maximum sporulation was observed in Samastipur (Pusa), Vaishali, Patna and Supaul isolates while minimum was observed in Rohtas, Bhojpur and Aurangabad isolates. Virulence of these isolates were also tested and those isolates which were highly sporulating were found most virulent but this was not true for all isolates. The virulent isolates established from this study can be used by plant breeders for screening varieties in pipeline against Brown spot disease which is the most threatening disease of Paddy in Bihar.

KEYWORDS:- Brown spot, *Bipolaris oryzae*, *Oryza sativa*.

INTRODUCTION:-

Rice is the second largest crop grown in the world in terms of both area and production. Rice is the staple food for more than half of world's population. However over 90 percent of the rice in the world is produced and consumed in Asian countries. Rice is known to be attacked by many diseases and some of them are seed borne. Brown leaf spot of rice caused by *Drechslera oryzae* (Breda de Haan) (Subram. and Jain) is one of the major fungal disease of rice which occurs in almost all the rice growing areas of the country (R.S. Singh., 2005).

This disease was responsible for the famous Great Bengal famine in India which led to death and migration of many people. This is also known as the disease of the poor farmers who are not able to provide all the required package of practices which a crop needs due to paucity of money. Most conspicuous symptoms of this disease occurs on leaves and glumes of maturing plants. Symptoms also appear on young seedlings and the panicle branches in older plants. Leaf spots may be evident shortly after seedling emergence and continue to develop until maturity. Leaf spots vary in size, are typically 1/8 inch in diameter, and are circular to oval in shape. The smaller spots are dark brown to reddish brown, and the larger spots have a dark brown margin and reddish brown to gray centers.

Bipolaris oryzae is a seed borne pathogen (Bedi and Dhaliwal, 1970; Fakir and Ahmed et. al. 1976 and Mian et. al. 1989). It can survive within the seed for four years.

Bipolaris oryzae was found to be the most predominant seed borne fungi by Mishra et.al (1988) when they studied samples collected from a different agroclimatic region of Bihar, Jammu, Andhra Pradesh and Orissa. Ali and Deka (1996) also reported the prevalence of *B. oryzae* from Assam. Diversity and pathogenicity of the rice brown spot pathogen has been investigated in Bangladesh by M M Kamal and M.A.T. Mia in 2009 using genetic fingerprint analysis.

MATERIALS AND METHODS:-

An extensive survey was conducted during kharif season of 2012 and 2013 in major rice growing areas of Bihar for collection of diseased samples of paddy (leaf as well as seed) to study morphological variability amongst the isolates of Brown spot fungus, *Bipolaris oryzae*. These samples from 25 districts were used for isolation of desired pathogen from spotted leaf and seeds with infection of brown spot disease. The disease was prevalent at all the locations surveyed and not even a single plant could be seen without brown spot symptoms in all the areas sur-

veyed. Amongst them the isolates from Samastipur, West Champaran, Aurangabad, Navada and Katihar which are geographically distantly located and with maximum disease incidence were selected for carrying out variability studies.

Affected tissues from the leaves were cut into small bits, washed thoroughly in running water to remove dirt. These were dipped in 0.1% mercuric chloride solution for 30-45 seconds. The bits were inoculated in PDA slant and incubated at $28 \pm 1^\circ\text{C}$ for 3-4 days. Spore suspension was made from seven days old cultures of the isolates. Out of fifty two isolates the five isolates from different geographical locations were designated as BO-Sam for Samastipur, BO-WC for West Champaran, BO-Aur for Aurangabad, BO-Naw for Nawada and BO-Kat for Katihar and were used to study various characters.

Morphological characters of all the five test isolates of *Bipolaris oryzae* like shape and size of spores were examined under calibrated compound microscope. Mean of 100 observations for each character was recorded. Characters like radial growth and sporulation of all isolates were also observed on PDA medium in Petridishes. Mycelial disc of 7 days old culture of the each isolate of the pathogen (5 mm diameter) were placed invertedly in the centre of Petridishes aseptically and incubated at $25 \pm 1^\circ\text{C}$. The observations for radial growth were recorded at 3 and 7 days intervals. For estimation of sporulation, number of spores was counted directly under compound microscope with the help of haemocytometer.

RESULTS AND DISCUSSION:-

It was found that the disease was prevalent in all the places surveyed. The disease incidence percentage varied from 15.30 to 52.10. Maximum disease incidence (52.10%) was recorded from Samastipur (Table 1). On the basis of colony morphology and growth pattern on PDA, these all isolates can be grouped into 5 categories: Black with fluffy growth (16 isolates), Black with suppressed growth (10 isolates), Grey

with cottony growth (9 isolates), Grey and white mix with cottony growth (12 isolates) and white with cottony growth (5 isolates). Based on colony diameter all these isolates can be grouped into 3 different categories: Slow growing (9 isolates), Moderate growing (15 isolates) and Fast growing (28 isolates). Spore dimensions of all these test isolates were also measured and the size varied from 5.34 micro meter to 7.48 micro meter in length while width varied from 4.10 to 5.51 micro meter. Minimum length and width was observed in BO-west champaran isolates while maximum length and width was observed in BO-Katihar isolates. Maximum sporulation was observed in Samastipur (Pusa), Vaishali, Patna and Supaul isolates while minimum was observed in Rohtas, Bhojpur and Aurangabad isolates. (Table 1.)

Colony diameter and growth behaviour of 52 isolates of *Bipolaris oryzae* were also studied which revealed that nine isolates from Gopalganj, Siwan Saran, Aurangabad, Rohtash and Bhojpur regions were slow growing and could attain a growth upto 45 mm only after seven days of inoculation on PDA. Twenty eight isolates from Samastipur, Muzaffarpur, Madhubani, Patna Begusarai, Saharsa, Darbhanga, Vaishali, Sitamarhi, and Buxar regions were very fast in growth and could attain a growth of 90 mm in only 4 days inoculated plates on PDA.

Virulence of these isolates were also tested on susceptible cultivar in pots and it was seen that the growth behaviour has no correlation with virulence. Few slow growing isolates were also found virulent while few fast growing isolates were less virulent. Spore count was also carried out for test isolates and the isolates which were highly sporulating were found most virulent but this was also not true for all isolates. Some of the less sporulating isolates were also found to be virulent. The virulent isolates established from this study can be used by plant breeders for screening varieties in pipeline against Brown spot disease which is the most threatening disease of Paddy in Bihar.

Table 1. Cultural characteristics of five test isolates of *Bipolaris oryzae* from Bihar

No. of groups	Test Isolates	Cultural characteristics	No. of Isolates in groups	Spore Size		Sporulation conidia/ml($\times 10^6$)
				Mean length	Mean width	
1.	BO-Sam	Black with fluffy growth	16	6.87	4.28	7.6 x 10 ⁶
2.	BO-WC	Black with suppressed growth	10	5.34	4.10	6.6 x 10 ⁶
3.	BO-Aur	Grey with cottony growth	9	5.47	4.44	6.9 x 10 ⁶
4.	BO-Naw	Grey & white mix with cottony growth	12	6.62	5.13	7.1 x 10 ⁶
5.	BO-Kat	White with cottony growth	5	7.48	5.51	7.3 x 10 ⁶

BO-Sam for Samastipur, BO-WC for West Champaran, BO-Aur for Aurangabad, BO-Naw for Nawada and BO-Kat for Katihar.

Table 2. Colony diameter and growth behaviour of 52 isolates of *Bipolaris oryzae* from Bihar

Places of collection	No. of Isolates	3 Days after Inoculation	7 Days after Inoculation	Growth Behaviour
Gopalganj, Siwan Saran, Aurangabad, Rohtash, Bhojpur,	9	10-20 mm	30-45 mm	Slow Growing
Sheohar , Supaul, West. Champaran, Jahanabad ,Gaya Nawada, Bhagalpur, Katihar, Purnia	15	30-40 mm	50-70 mm	Moderate Growing
Samastipur , Muzaffarpur, Madhubani, Patna Begusarai, Saharsa, Darbhanga, Vaishali, Sitamarhi, Buxar	28	75-80 mm	90 mm (4 days)	Fast Growing

ACKNOWLEDGEMENT:

The author is thankful to the Department of Science and Technology , GOI for financial support and Head IARI Regional Station Pusa (Bihar) and Head DSSST ICAR-IARI for providing necessary facilities for carrying out research work.

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