Survey, Collection and Isolation of Isolates of *P. oryzae* causing rice blast across different zones of Karnataka

***[1]B. Manjunatha and [2]M. Krishnappa**

[1&2] Department of Applied Botany, Kuvempu University, Shankaraghatta, Shivamogga, Karnataka, India

Abstract:

A survey was conducted during kharif 2016-17 in different zones of Karnataka to assess the per cent severity and incidence of rice leaf (Pyricularia oryzae) and neck blast. The freshly infected leaves were randomly collected from all the zones viz., Northern dry zone (Zone-3), Southern dry zone (Zone- 6), Southern transitional Zone (Zone-7), Northern transition zone (Zone-8) and Hilly zone (Zone-9) of Karnataka from these samples the blast disease causal organism (P.oryzae) was isolated by following tissue isolation method and monoconidial isolation. Among all the zones, the highest per cent disease severity of the rice leaf blast was recorded (49.97 %) in Hilly zone (Zone-9) and lowest incidence was recorded (30.23) in Southern dry Zone. Among all the zones, the highest per cent disease incidence of neck blast was recorded (20.68 %) in Hilly zone (Zone-9) and lowest incidence was recorded in (11.00 %) in Southern dry zone 6.

Key words: Blast, Pyricularia, Incidence, Survey, Paddy, Zone

Rice (*Oryza sativa* L.) is one of the most important cereal crop of family Poaceae. It is a staple food crop of 60 per cent of the world's population. Rice is one of the diverse crop grown in different agro-climatic conditions. Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The common wealth mycological Institute has recorded its presence from 85 countries throughout the world. The disease is adaptable to adverse environmental conditions of widely fluctuating temperatures and relative humidity. It appears in irrigated low land or rainfed upland rice as well as in submerged or deep water rice (Anon., 1963). Blast is caused by *Magnaporthe grisea* (Hebert) Barr) (Syn: *Pyricularia grisea* (Cooke) Sacc.) filamentous ascomycetes fungi infecting more than 50 hosts. Infection occurs on leaves during vegetative phase and on panicles and neck during reproductive phase of the crop resulting in significant loss in yield and grain quality. Blast causes heavy loss in yield which varies from place to place. The pathogen is known for its high genetic plasticity. Due to continuous shift in its genetic makeup resistant varieties are often succumb to infection. An understanding of population structure and virulence spectrum of the pathogen is essential for the successful implementation of disease management strategies. The dynamic evolution of blast pathogen in response to rice genotypes often complicates breeding for blast

resistance. Owing to its changing virulence composition, the present study was initiated to understand the extent of variation among the isolates collected from different rice growing zones of the Karnataka.

Material and methods:

Roving survey was conducted during *kharif* 2016-17, across different zones of Karnataka to assess the per cent severity and incidence of rice leaf and neck blast according to standard disease scale (Table 1).

Collection of Infected Specimen and Isolation of the Fungus:

The freshly infected leaves were randomly collected from all the zones of Karnataka and were used for isolation by adopting the following methods.

Tissue Isolation Technique

Rice leaves with blast symptoms were first washed in tap water, and then cut into small bits of 2mm size, containing the blast lesion along with a portion of healthy tissue surrounding the lesion. These bits were surface sterilized with 0.1 percent sodium hypochlorite solution for two min, followed by three changes of sterilized distilled water. These bits were transferred into petri dishes containing 15 ml solidified PDA under aseptic conditions, and incubated at 28 ± 1^0C and watched for the growth of colony, regularly. For the growth of the pathogen from the tissue, or for any contamination, up to fourteen days investigation was done. After fourteen days of incubation, a small loop of fungal culture from the colonies was picked, and put on a clean slide containing a drop of lacto phenol. The slide was observed under low and high power objectives for the presence of pyriform conidia.

Single Spore Isolation Technique (Monoconidial Isolation)

Well-developed lesions were identified, excised and washed in running tap water. The leaf bits were surface sterilized with 0.1 sodium hypochlorite for 2 min, and then washed three times with sterile water and allow for speculation on sterilized glass slides, by incubating in a moist chamber at 28 ± 1^0C for 48 h. Well sporulated lesions were placed in double distilled water, in the test tubes and vortexed for 1 min. About 1 ml of spore suspension was added to sterilize plates, and 2% agar was added. Single spore was located and picked up microscopically. Each spore was eventually transferred to solidified PDA slants. The slats were incubated at 28 ± 1^0C for 2 days and stored at 4^0C for further use. (Goh,1999).

Results and discussion

Survey on Severity of Rice Leaf and Neck Blast (*P. Oryzae*)

Roving survey was conducted during *kharif* 2016-17 around the different Zones of Karnataka to assess the per cent severity of blast disease of rice and the results of the data is presented in table 2. The symptoms in the field of rice were recognized by broadly spindle

shaped spots, with the pale ashy centre and brownish red margins seen on the leaf. In case of severe infection, several such spots coalesce and the lamina are destroyed (Plate 1) Average per cent severity of rice leaf blast i.e. 39.27 per cent was recorded across five zones. Highest per cent disease severity was recorded (49.97 %) in Hilly zone (Zone -9) and the lowest was recorded (30.23 %) in Southern dry Zone (Zone-6) (fig.1). Average per cent severity of rice Neck blast i.e. 15.45 per cent was recorded across five zones. Highest per cent disease severity was recorded (20.68 %) in Hilly zone (Zone -9) and the lowest was recorded (11.00 %) in Southern dry Zone (Zone-6) (Table 2, fig 2).

Collection, Isolation and Identification of *P. Oryzae*

The infected leaf and neck blast samples having typical symptoms of blast on rice crop caused by *P. oryzae* from various zones of Karnataka (Plate 1). From these samples, the causal organism was isolated by following tissue isolation method. Further, monoconidial isolation was done to obtain pure cultures of all isolates, as detailed in 'material and methods. The fungus was sub-cultured on PDA slants, and stored in refrigerator at 4^0C. For further studies, the original culture of each isolates was revived once in 30 days.

Survey, Isolation and Proving Pathogenicity of *P. Oryzae*

A survey of the disease prevalence of rice blast was undertaken in different locations across five Zones of Karnataka *viz*., Northern dry zone (Zone-3), Southern dry zone (Zone- 6), Northern transition zone (Zone-8) and Hilly zone (Zone-9). Infected samples showing typical blast symptoms on leaf and neck portion were collected. The culture of each location was considered as an individual isolates. During survey, highest average per cent leaf blast disease severity (68.23%) was recorded in Zone -9 (Hilly zone) in the variety HR-12 whereas, lowest percent severity (26.50%) was recorded in the Zone -8 (Northern transitional zone). During survey, highest average per cent neck blast disease severity (26.20%) was recorded in Zone -9 (Hilly zone) in the variety Intan whereas, lowest per cent severity (11.00%) was recorded in the Zone -6 (Southern transitional zone) (fig.3). Morgan Hossain (2000) and Anwar *et al.*, (2009) also surveyed for the per cent severity of rice blast. Morgan Hossain (2000) recorded 61.66 per cent disease incidence in Uttar Kannada due to blast caused by *P. oryzae*. However, the present study shows that the maximum leaf blast (68.23%) neck blast disease severity (26.50 %) were recorded in Zone-9 and Zone-6. Hence, the variation in disease incidence among different talks is attributed to the varied climatic conditions and edaphic factors like soil temperature, soil moisture and soil pH (Akhilesh *et. al*. 2017).

The sample of rice leaves affected by blast disease were collected from different zones of Karnataka. The pathogen isolated from diseased leaf showing typical symptoms was identified, as *P. oryzae* on the basis of morphological characters. Pure culture was maintained in PDA medium through hyphal tip isolation technique. The isolation of rice blast fungus observed were in agreement with the description, Dhua (1986), Xia et al., (1993), Goh (1999) and Lima and Duclos (2001). The symptoms of rice blast observed were in agreement with the description of Ghose *et al.,* (1960); Pinnschmidt *et al.,* (1994); Padmanabhan (1974) and Manibhushan Rao (1994). Hence, pathogencity tests showed

typical symptoms, when inoculated with pathogenic culture of *P. oryzae*. Isolation of the fungus from such affected leaf tissue in variably yielded pathogenic culture of *P. oryzae.* Hence, the present study clearly indicated that *P. oryzae* is involved in causing blast disease in rice.

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Table 1: Standard screening scale (0-9 grade for disease rating) was used for blast disease of rice (Source-IRRI,1996; Ghazanfar *et al*., 2009)

Grade	Disease severity	Host response
0	Lesion are not present	Highly resistant (HR)
1	Small brown specks of pin point size on lower leaves	Resistant (R)
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin. Lesions are mostly found on the lower leaves	Moderately resistant (MR)
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Moderately resistant (MR)
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately susceptible (MS)
5	Typical susceptible blast lesions of 3 mm or longer infecting 4-10% of the leaf area	Moderately susceptible (MS)
6	Typical susceptible blast lesions of 3 mm or longer infecting 11-25% of the leaf area	Susceptible (S)
7	Typical susceptible blast lesions of 3 mm or longer infecting 26-50% of the leaf area	Susceptible (S)
8	Typical susceptible blast lesions of 3 mm or longer infecting 51-75% of the leaf area many leaves are dead	Highly susceptible (HS)
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly Susceptible (HS)

Table 2: Percent Severity and Incidence of Rice Leaf and Neck Blast Disease across different zones of Karnataka

Zone	Location	Variety	Leaf blast (%)	Mean (%)	Neck blast (%)	Mean (%)
Northern Dry Zone 3	Gangavathi	BPT-5204	35.66	33.94	15.20	14.60
	Siraguppa	BPT-5204	32.22		14.00	
Southern Dry Zone 6	Mandya	Tanu	30.23	30.23	11.00	11.00
Southern Transition Zone 7	Shivamogga	Jyothi	36.55	37.53	12.50	12.86
	Shivamogga	Abhilash	38.52		13.22	
Northern Transition Zone 8	Haveri	Abhilash	26.50	44.70	14.20	18.13
	Mugad	Abhilash	62.40		18.20	
	Mugad	Intan	45.22		22.00	
Hilly Zone 9	Sirsi	Intan	48.53	49.97	22.50	20.68
	Malagi	Abhilash	38.50		19.20	
	Malagi	Intan	42.23		18.20	
	Mundogadu	Intan	44.56		16.30	
	Mudigere	Intan	45.23		18.20	
	Ponnampet	HR-12	68.23		24.20	
	Ponnampet	Intan	62.56		26.20	

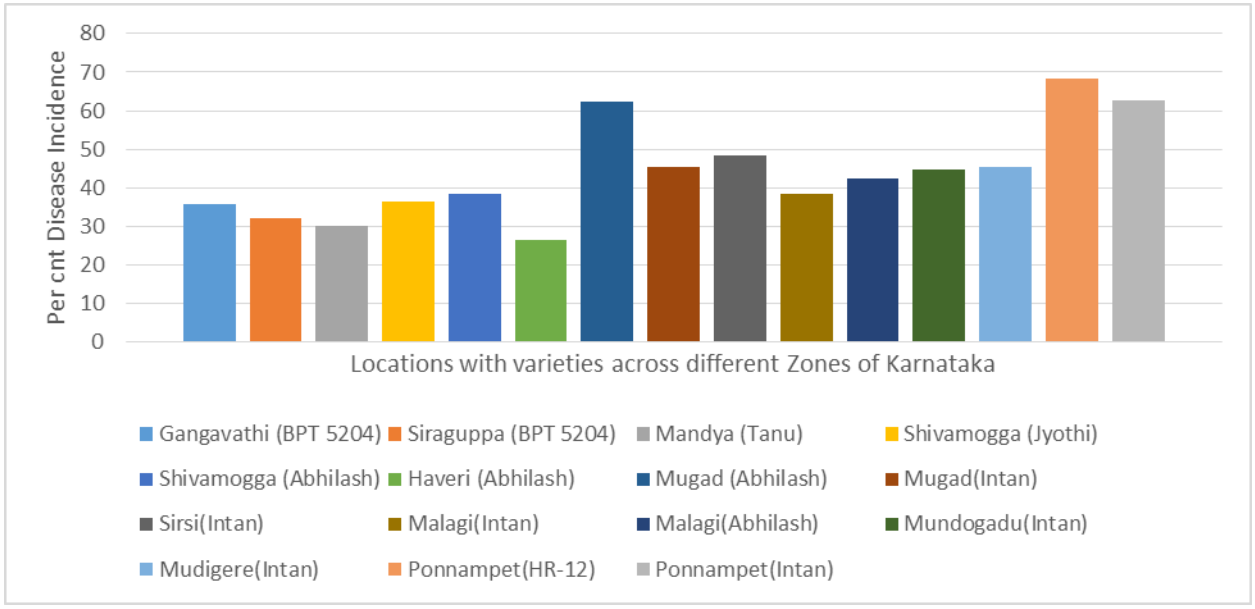

Figure 1: Per Cent Disease Severity of Rice Leaf Blast of different Zones of Karnataka

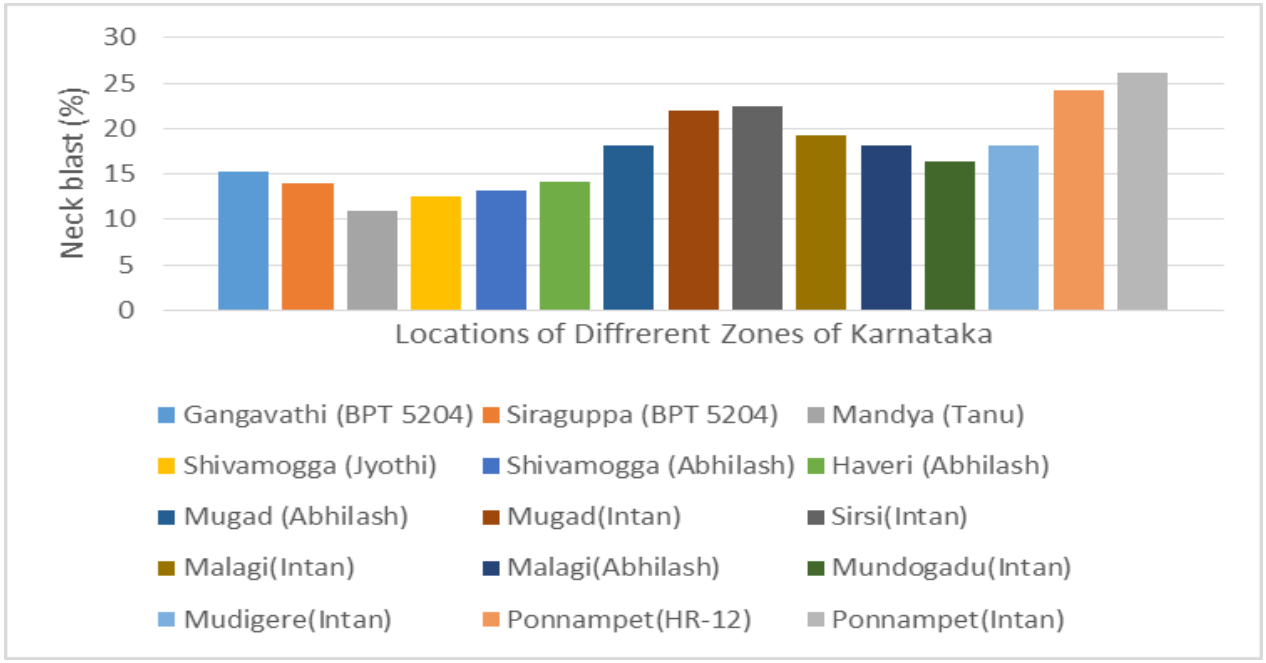

Figure 2: Per Cent Disease Severity of Rice Neck Blast of different Zones of Karnataka

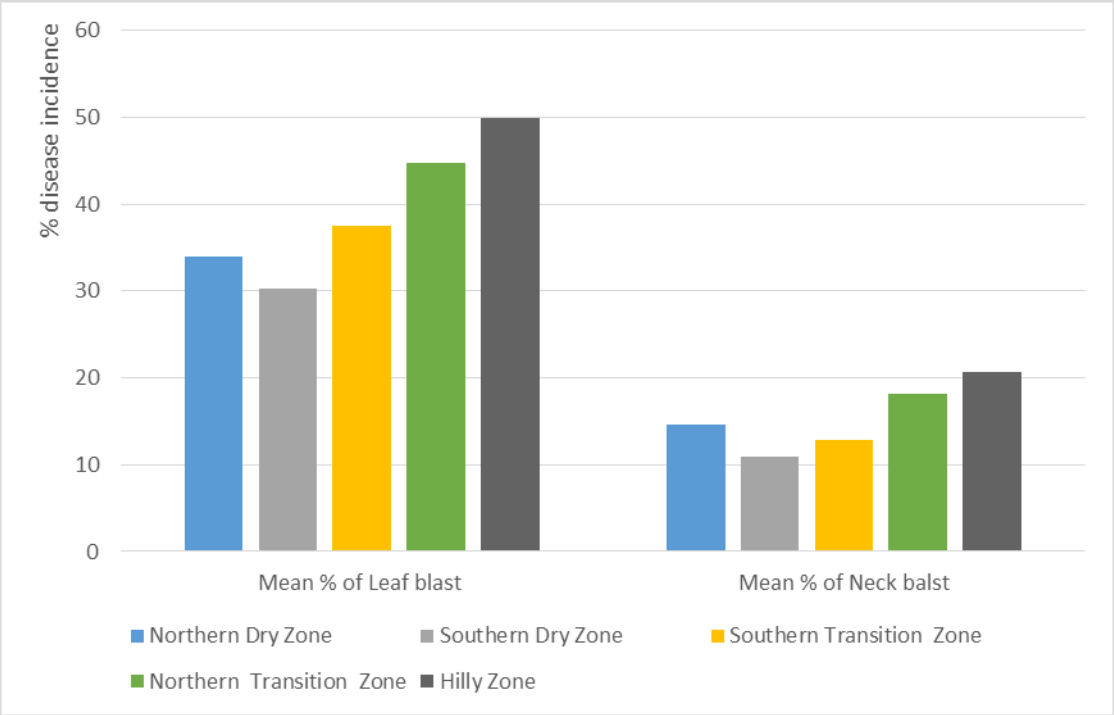

Figure 3: Mean per cent of Disease Severity of Rice Leaf and Neck Blast of different Zones of Karnataka

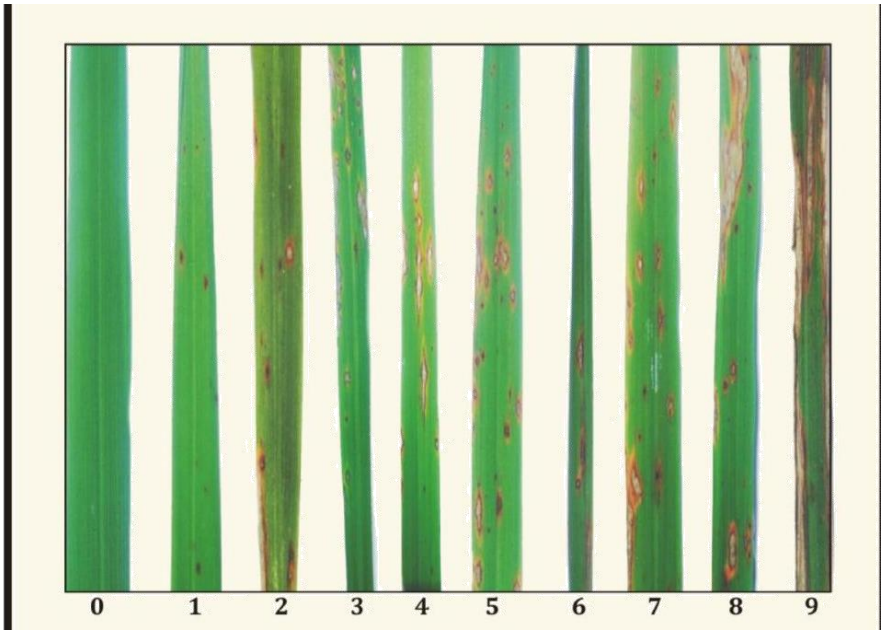

Plate 1: 0-9 scale indication of diseased leaves of rice caused by *P.oryzae*