Research Article

MORPHOLOGICAL VARIABILITY AMONG VARIOUS ISOLATES OF *MAGNAPORTHE GRISEA* COLLECTED FROM PADDY GROWING AREAS OF KASHMIR

***Mohd. Shahijahan Dar, Sajad Hussain, Afshana Bashir Darzi and Snober H. Bhat**
Department of Botany, Govt. Degree College Pulwama (J&K) 192301- India.
*Corresponding author's E-mail: drjahan96@gmail.com

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ABSTRACT

Various isolates of *Magnaporthe grisea* were collected from different diseased plant components (leaf, node, neck, seeds and rachis) at different locations of various paddy growing areas of Kashmir to assess the morphological variability among them. Data on morphological variability revealed that the isolates from leaf component MG_1, MG_3, MG_5, MG_{10} and MG_{11} showed excellent sporulation and growth on Oat meal agar (OMA) and Rice decoction agar (RDA) medium, while the isolate MG_1 from high altitude area showed extensive aerial growth with formation of sclerotia in culture which possibly acts as a source of perpetuation for the fungus in the areas with low temperature regimes. Excellent sporulation and growth of the isolates of nodal components viz; MG_4 and MG_7 were observed on Rice decoction agar (RDA) medium and growth on other media was not encouraging. The isolates from neck component viz; MG_2, MG_6 and MG_{12}, showed effuse and slow growth, greyish to pale olive in colour and the isolate from rachis MG_8 exhibited submerged and thin growth but in concentric rings, light grey in colour, conidia being hyaline in colour and developed by both blastic as well as gangliar fashion. The isolate MG_9 from seeds showed growth in concentric circular rings but submerged greyish brown in colour. On media preferences, the isolate grows profusely and sporulates well on Oat meal agar (OMA). The results showed considerable morphological and physiological variability among the isolates in different growth media used in the present study.

Keywords: Morphological variability, Isolates, *Magnaporthe grisea*, Paddy, Diseased components.

INTRODUCTIN

In India, the productivity of rice (*Oryza sativa* L.) is less than those in agriculturally advanced countries because of poor agronomic practices followed in many remote areas and partially because a huge amount of crop being damaged by abiotic and biotic stresses (Garret, 1965)[1]. A major constrain in profitable rice production is the occurrence of the certain fungal diseases and paddy blast is one of the most important disease of rice worldwide. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia grisea* Sacc. (*Pyricularia oryzae* Cavara (= teleomorph *Magnaporthe grisea* (Hebert) Barr Comb nov.). Due to continuous and extensive cultivation of rice in Kashmir division, blast disease caused by *(M. grisea*) has been recognized as the most devastating and damaging disease causing major problem of rice production. Losses due to the blast disease may range up to 90% depending upon the component of the plant infected. Total destruction of the crop over large areas has been reported from Jammu and Kashmir (Padmanabhan, 1963)[2]. *Magnaporthe grisea* may infect most above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality.

MATERIALS AND METHODS

Diseased plant components (leaf, node, sheath, neck, seeds and rachis) of surveyed samples from farmer's field were kept in the BOD for incubation at 24 hrs, and then the pathogen (*Magnaporthe grisea*) was isolated from the collected materials during the crop season. Well-developed susceptible lesions were identified, excised and washed in running water for two hours. The bits of plant components were surface sterilized with mercuric chloride (0.1%) for three seconds, then serially washed with sterile water and put in moisture chamber at 28°C for four hours. Well sporulated lesions were placed in double distilled water in test tubes and vertexed for one minute. About one ml spore suspension was added into sterilized plates already poured agar medium. Single spores were located, picked up along with the medium under microscope and transferred to potato dextrose agar slants. The slants were incubated at 28^0C for the profuse growth of the fungus for two days and then identified and confirmed the pathogen on the physiological and morphological basis on Potato dextrose agar, Oat meal agar, and Rice decoction agar medium and also evaluated the isolates such as MG1, MG2, MG3, MG4, MG5, MG6, MG7, MG8, MG9, MG10, MG11 and MG12 against various temperatures viz; 10, 15, 20, 25 and 30°C for growth characteristics. Three replications were maintained of each treatment. After two weeks of inoculation, the radial mycelial growth of the fungus was measured.

Table 1: Morphological variability among the various isolates of *Magnaporthe grisea* collected from the paddy growing areas of the Kashmir.

S. No	Isolates	Components	Cultural characteristics				Conidiophore	Conidial formation			Conidial morphology			Sclerotial like formation
			Growth on medium			Colony character		Blastic	Gang-liar	Both	Colour	Septation	Shape	
			RDA	PDA	OMA									
1	MG1	Leaf	++ (a)	++ (c)	+++ (a)	Aerial, white, characteristic brownish powdery mass in the centre	Fasciculate, slightly constricted at septa. Monosporic first then pleurogenous on sympodium	+	+	+	Hyaline	2	Pyriform, protruding hilum at base.	+
2	MG2	Neck	++ (b)	+ (c)	++ (a)	Effuse, grayish brown, mycelium immersed.	Macronematou, unbranched, geniculate towards apex.	+	+	+	Pale olive	2	Pyriform to obclavate, base rounded, apex narrowed.	+
3	MG3	Leaf	++ (a)	+ (c)	+++ (a)	Mycelium submerged, olivaceous, brown, medi turning with advancement of growth	One to many, fasciculate, lighter towards apex	+	+	+	Hyaline to pale olive	2	Solitary, smooth, slightly constricted at septa	+
4	MG4	Nodal	+++ (a)	+ (c)	++ (b)	Effuse, rallies formation from the centre of colony, light grayish.	Single or fascicles, growth sympodial, grayish in colour.	+	+	+	Hyaline to pale olive.	2	Pyriform, no hilum at the base.	+
5	MG5	Leaf	++ (a)	++ (b)	+++ (a)	Thin, hairy, aerial light grey.	Fasciculate, greyish in colour.	+	+	+	Hyaline	2	Narrowly pyriform to obclavate with rounded base and narrowed towards tip, protruding hilum at the base.	+
6	MG6	Neck	++ (b)	+ (c)	++ (a)	Colony effuse, grayish brown.	Unbranched, geniculate towards apex.	+	+	+	Pale olive	2	Pyrifom, smooth with pointed tip.	+
7	MG7	Nodal	+++ (a)	+ (c)	++ (b)	Effuse, submerged, media turning black with age, formation of rallies from the centre of colony.	Fasciculate, sympodial growth, no constrictions at septa.	+	+	+	Pale olive	2		+
8	MG8	Rachis	+++ (a)	++ (c)	++ (a)	Growth submerged and thin but in concentric rings, light grayish in colour	Dark colour but lighter towards apex. Slightly constricted at septa. Base swollen.	+	+	+	Hyaline	2		+
9	MG9	Seeds	++(b)	++(c)	+++(a)	Growth in concentric rings but submerged, grayish brown.	Slender, unbranched, flexuous geniculate towards apex, pale brown.	+	+	+	Hyaline to pale olivaceous	2		+
10	MG10	Leaf	+ (c)	+ (c)	++ (b)	Mycelium thin, appressed to the medium, light grayish in colour and only central part of colony turns greyish black.	Fasciculate, sympodial growth. Constriction at septa.	+	+	+	Hyaline	2		+
11	MG11	Leaf	++ (b)	++ (b)	++ (a)	Mycelium aerial, light grey in colour.	Simple fasciculate, unbranched, sympodial growth.	+	+	+	Hyaline	2		+
12	MG12	Neck	++ (b)	+ (c)	++ (a)	Colony effuse, thin hairy, olivaceous brown.	Unbranched, geniculate towards apex. Pale brown.	+	+	+	Pale olive	2		+

MG = *Magnaporthe grisea*, + = Good, ++ = Medium, +++ = Excellent RDA = Rice decoction Agar, PDA = Potato dextrose Agar, OMA = Oat meal Agar

RESULTS AND DISCUSSION

Morphological variability among various isolates of *Magnaporthe grisea* from different locations and different components of rice plants studies revealed that considerable morphological and physiological variability exists between the isolates investigated. The isolates from leaf component MG_1, MG_3, MG_5, MG_{10} and MG_{11} showed excellent sporulation and growth on Oat meal agar (OMA) and rice decoction agar (RDA) medium, however the isolate MG_1 from high altitude area has extensive aerial growth with formation of sclerotia in the culture which possibly acts as a source of perpetuation for the fungus in the areas with low temperature regimes. The conidia being hyaline, with gangliar formation, two septate, pyriform. The isolates from neck component viz; MG_2, MG_6 and MG_{12}, the growth is effuse and slow, greyish to pale olive in colour. The isolates of nodal components viz; MG_4 and MG_7 exhibited excellent growth and sporulation on rice decoction agar (RDA) medium but growth on other media was not encouraging. The growth

pattern was effuse with formation of rallies from the centre of the colony, light greyish in colour but no sclerotial formation and media turns black with age. The formation of conidia is both blastic and gangliar, the colour being hyaline to pale olive, pyriform in shape with two septation with no hilum at the base. The isolate from rachis MG_8 exhibited submerged and thin growth but in concentric rings, light gray in colour, conidia being hyaline in colour and developed by both blastic as well as gangliar fashion. The isolate MG_9 from seeds showed growth in concentric circular rings but submerged greyish brown in colour. On media preferences, the isolate grows profusely and sporulates well on Oat meal agar (OMA). The conidia formation being blastic, the conidia are two septate pyriform to obclavate with basal appendages. (**Table-1**)

The present investigation revealed that the isolates of *Magnaporthe grisea* differ in cultural morphology and with the medium used. Similar trend was observed by Leaver *et al*. (1947)[3] who strongly suggested that presence of growth promoting substance in the rice straw extract which stimulates growth and sporulation and found biotin and thiamine to be necessary for growth. Similar results were obtained by Tanaka and Katsuki (1951a, b)[4-5] and Otani (1952b)[6]. Otsuka *et al*. (1957d, 1958b)[7-8], working with 47 isolates of the fungus, also found that a few isolates could grow in biotin- deficient media and a few others in thiamine- deficient media. The results are in line with the opinion of Tanka *et al*. (1956)[9] who reported that succinic, malic and citric acids, known as the components of TCA cycle and contained in rice leaves, were excellent additive stimulants. Similar results were ascertained by many workers (Sawada, 1917; Nisikado, 1926; Kulkarni and Patel, 1956; Ono and Nakazato, 1958)[10-11-12-13] and have observed that conidia produced on culture media to be longer than those on the host plants and to very according to the kind of medium which are in line with our investigation. Our results are in line with Hussain *e al* . (2004)[14] who reported that potato dextrose agar was found best support for growth of *P.grisea*, Similarly Awoderu (1990)[15] had already reported best growth of *P.grisea* on PDA. Simultaneously they have also observed that significantly more growth of the fungus occurred at 30^0C while PH in range of 6.0 to 7.0.

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