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Impact of nutritional sources on vegetative growth and conidial formation of *Magnaporthe oryzae* Couch

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Abstract:

In a laboratory experiment, different carbon sources viz., glucose, dextrose, sucrose, maltose, starch, and cellulose at 10,000 ppm, 20,000 ppm and 30,000 ppm and nitrogen sources viz., NPK, NP, DAP, Urea and Sodium nitrate at 10,000 ppm, 1000 ppm and 100 ppm were used to evaluate their effects on vegetative growth and sporulation of Magnaporthe oryzae (syn: Pyricularia oryzae Cav.). Among carbon sources, significantly highest mycelial growth was took place on the medium amended with higher concentrations of glucose followed by 30,000 ppm cellulose, starch and 20,000 ppm of maltose. It was also observed that M. oryzae was also capable to grow in the absence of any carbon source, as 36.7 mm colony growth of M. oryzae was obtained on the medium without any carbon source. Maximum conidial production was observed on medium amended with 30,000 ppm of dextrose followed by 20, 000 ppm dextrose and 30,000 ppm

glucose. Among different nitrogen sources, maximum colony growth of M. oryzae was recorded at 10,000 ppm of Sodium nitrate followed by 10,000 ppm of Urea and NPK, and 1000 ppm of DAP. The sporulation of M. oryzae was greatly influenced by the type of nitrogen source used. Generally, DAP and Urea significantly favoured the conidial formation; whereas, conidial production was significantly reduced in medium amended with NPK, NP and Sodium nitrate. Maximum number of conidia were recorded in medium amended with 1000 ppm of urea followed by 1000 ppm and 10,000 ppm of DAP.

Key words: Rice, *Magnaporthe oryzae,* carbon, nitrogen, vegetative growth and sporulation

Introduction

Magnaporthe oryzae (syn: *Pyricularia oryzae* Cav.) (Class: Sordariomycetes, Order: Magnaporthales, Family: Magnaporthaceae) is a filamentous, ascomycetous fungus and one of major fungal pathogen of rice causing rice blast disease, which is distributed worldwide and prevailing in more than 85 countries of the world (Scardaci et al., 1997; Gilbert et al., 2004). Rice blast can be appeared at any stage of growth and produced various symptoms (Izadyar, 1985). On the leaf, fungus produced diamond shaped spots which are grayish or white with brown or reddish brown margins (Hajano et al., 2011). An infected node became black and finally collapsed. Fungus can also infect flag leaf by producing lesions which are grayish coloured.

All fungal species required certain carbon and nitrogen sources for their vegetative growth and sporulation. The size and shape of spores and colonies of filamentous fungi are the important factors in fungal identification. Various types of carbon and nitrogen sources are utilized by different species depending upon their ability in secreting various enzymes for the degradation of the polymers into small molecules (Lee et al.,

2007). This means that, the type and concentration of carbon and nitrogen sources, carbon/nitrogen ratio are the important factors in medium formulation for the enhancement of fungal growth and sporulation. Therefore, several studies have been carried out to determine the optimum carbon and nitrogen requirements of various fungi (Haq et al., 2001; Hossain et al., 2004; Khanzada et al., 2006; Tripathi, 2006; Kumara and Rawal, 2008; Sangeetha and Rawal, 2008). Keeping in view the economical importance of rice blast fungus, the present study was carried out to determine its nutritional requirement with especial reference to impact of varied carbon and nitrogen sources on the vegetative growth and development of conidia of *M. oryzae* under *in-vitro* condition.

Materials and Methods

Source of Test Fungus: During previous study *M. oryzae* was isolated from diseased leaves of rice varieties collected from district Badin, Sindh during 2010 (Hajano et al., 2011).

Effect of different carbon sources on mycelial growth and sporulation of *M. oryzae*: Different carbon sources such as glucose, dextrose, sucrose, maltose, starch, and cellulose at 10,000 ppm, 20,000 ppm and 30,000 ppm were added to potato agar medium before the medium was autoclaved. Potato agar medium without carbon sources served as control. Before pouring of sterilized medium in Petri dishes, streptomycin sulphate at $1mlL^{-1}$ medium and penicillin at 1000,000 $unitsL^{-1}$ were used as antibiotic to avoid bacterial contamination. After solidifying of the medium, one cm disc of pure culture was placed in the center of Petri dishes and incubated at 30^0C. There were five replications of each treatment. Diameter of growing colonies was recorded in mm after each 24 hour till the plates were filled in either treatment. Conidia were counted after 15 days of inoculation. For this purpose, three discs of 5

mm diameter of culture were harvested from different locations randomly from each Petri dish. Each disc was placed in test tube containing 1 ml sterilized water and shake thoroughly to detach the conidia from the growing culture. The prepared solution was placed in hemocytometer with the help of pipette for counting conidia, it was repeated three times.

Effect of different nitrogen sources on mycelial growth and sporulation of *M. oryzae*: Different nitrogen sources such as NPK, NP, DAP, Urea and Sodium nitrate at 10,000 ppm, 1000 ppm and 100 ppm were added to potato agar medium after sterilization at the time of pouring. Potato dextrose agar medium without nitrogen sources served as control. Before pouring sterilized medium in Petri dishes, streptomycin sulphate at $1mlL^{-1}$ medium and penicillin at 1000,000 $unitsL^{-1}$ were used as antibiotic to avoid the contamination of bacteria. After solidifying of the medium one cm disc of pure culture was placed in the center of Petri dishes and incubated at 30^0C. There were five replications of each treatment, diameter of growing colonies was recorded in mm after each 24 hour of inoculation, while conidia were counted after 15 days of inoculation, as described previously.

Statistical analysis: Data was analyzed by ANOVA using Statistix 8.1 software. Least significant differences (LSD) were calculated using significant level at $P = 0.05$.

Results

The isolated fungus was identified on the basis of its conidial morphology. *Magnaporthe oryzae* developed grayish coloured colonies on PDA medium. Conidia were 2-3 celled which are pyriform in shape and produced on the tip of septate conidiophores.

Effect of different carbon sources on mycelial growth and sporulation of *M. oryzae*: There was no active response of *M. oryzae* observed, against various carbon sources used with different concentrations. Mycelial growth was significantly highest on the medium amended with medium concentrations of glucose (20,000 ppm), followed by glucose and cellulose 30,000 ppm as compare to control (Fig. 1). The results also indicated that *M. oryzae* was also capable to grow without any additional amendment carbon source, as 36.7 mm colony growth of *M. oryzae* was obtained on the medium without any carbon source (control) (Fig. 1).

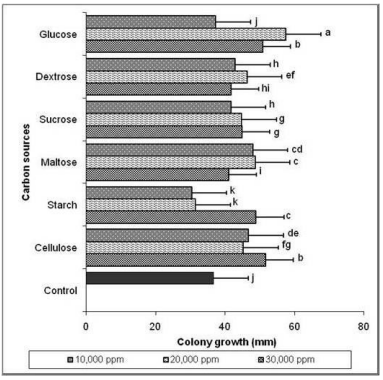

Fig. 1. Effect of different carbon sources on mycelial growth of *Magnaporthe oryzae*.
Means followed by different letters in respective bar are significantly different at $P = 0.05$.

In contrast to mycelial growth, conidial production of *M. oryzae* was gradually influenced by the type and quantity of carbon sources used. The maximum number of conidia formed at 30,000 ppm of dextrose (165.55) followed by 20, 000 ppm dextrose (151.00), and 30,000 ppm glucose (124.10), respectively (Fig. 2). The other carbon sources amended in basic medium significantly enhanced the conidial formation as compared to un-amended medium (control) which produced minimum number of conidia (Fig. 2).

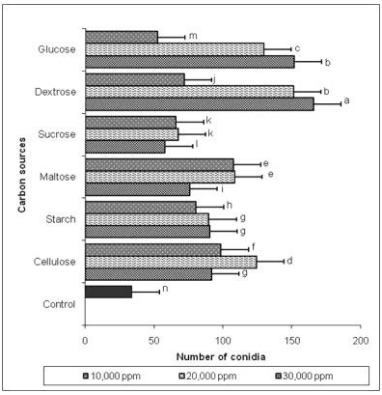

Fig. 2. Effect of different carbon sources on sporulation of *Magnaporthe oryzae*.

Means followed by different letters in respective bar are significantly different at $P = 0.05$.

Effect of different nitrogen sources on mycelial growth and sporulation of *M. oryzae*: The maximum colony growth of *M. oryzae* was recorded at 10,000 ppm of Sodium nitrate (51.4 mm) followed by 10,000 ppm of Urea (51.3 mm), 10,000 ppm of NPK (51.24 mm) and 1000 ppm of DAP (51.0 mm) (Fig. 3). There was no significant difference in mycelial growth obtained at lower concentrations of various nitrogen sources and that in un-amended PDA medium (44.4 mm) after 5 days of incubation (Fig. 3).

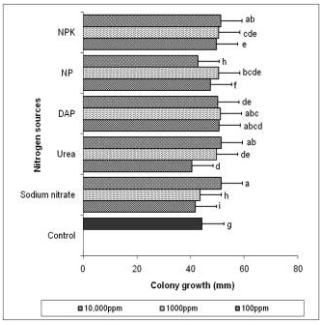

Fig. 3. Effect of different nitrogen sources on mycelial growth of *Magnaporthe oryzae*.

Means followed by different letters in respective bar are significantly different at $P = 0.05$.

The sporulation of *M. oryzae* was greatly influenced by the type of nitrogen source used. The conidia were significantly highest at 1000 ppm of Urea (227.30) followed by 1000 ppm and 100 ppm of DAP which produced 212.77 and 199.00 conidia, respectively (Fig. 4). The lowest number of conidia was observed at 1000 ppm of Sodium nitrate (56.87) followed by 100 ppm of NPK and NP with 59.33 and 73.40 respectively (Fig. 4). In general, DAP and Urea significantly favoured the conidial formation of *M. oryzae*, whereas conidial formation was significantly reduced in medium amended with NPK, NP and Sodium nitrate (Fig. 4).

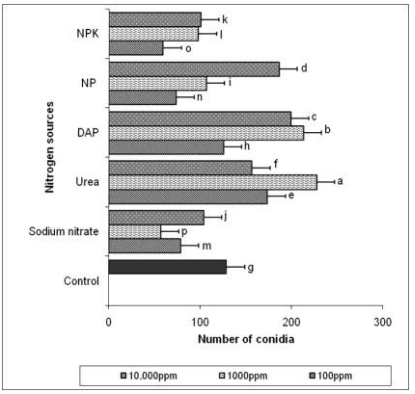

Fig. 4. Effect of different nitrogen sources on sporulation of *Magnaporthe oryzae*.

Means followed by different letters in respective bar are significantly different at $P = 0.05$.

Discussion

Plant pathogen have higher rate of mutation. Due to mutation they also can alter their metabolic and sporulation capability. Therefore, favourable nutiritional condition of a particular pathogen is essential to know. In this study, we evaluated the effect of different carbon and nitrogen sources on the mycelial growth and condial production of *M. oryzae*. All the tested carbon sources increased mycelial growth, but conidial

production showed specificity to different carbon sources and also to the concentrations of carbon. Nitrogen sources also showed similar trend that mycelial growth of *M. oryzae* was slightly influenced as compare to conidial production on these nitrogen sources. These results are in agreed with previous reports that growth and development of *M. oryzae* was influenced by different carbon and nitrogen sources. Tripathi (2006) found that *M. oryzae* grew best on maltose followed by glucose and barium nitrate amended medium. Hossain et al., (2004) observed that starch gave the maximum dry mycelial weight and asparagine is best as nitrogen source of *M. oryzae*. Similarly, Awoderu et al., (1991) reported that ammonium chloride favoured mycelial growth and conidial production of *M. oryzae*.

However, glucose and Sodium nitrate were proved as efficient carbon and nitrogen sources for mycelial growth of *M. oryzae*. It indicates that enzymatic system of *M. oryzae* was effective to break molecules of glucose and Sodium nitrate for its development as compare to other sources. Enzymatic system of fungi has been shown as major factor for breaking molecules for their development (Lopez et al., 2003). Whereas, maximium *M.* oryzae conidia were produced with amendment of dextrose and Urea. These result showed that conidial production is not always correlated with the mycial growth.

REFERENCES

Awoderu VA, Esuruoso OF and Adesoun OO, "Growth and conidia production in race NG-5/IA-65 of *Pyricularia oryzae* Cav. *in-vitro*," *Journal of Basic Micro Biology* 31 (1991): 163-168.

Gilbert MJ, Soanes DM and Talbot NJ, "Functional genomic analysis of the rice blast fungus *Magnaporthe grisea*," *Applied Mycology and Biotechnology* 4 (2004): 331-352.

Hajano J, Pathan MA, Rajput AQ and Lodhi AM, "Rice blast-mycoflora, symptomatology and pathogenicity," *IJAVM*. 5 (2011): 53-63.

Haq I, Ali S, Ashraf H, Butt WA, Qadeer MA, Shafiq K and Iqbal J, "Effect of mineral nutrients on the biosynthesis of citric acid by *Aspergillus niger* UV-6, using sucrose salt media," *Pakistan Journal of Botany* 33 (2001): 535-540.

Hossain MM, Srikant K and Hegde YR, "Physiological and nutritional studies on *Pyricularia grisea*, the causal agent of blast of rice," *Karnataka Journal of Agricultural Sciences* 17(2004): 851-853.

Izadyar M, "Comparison of effectiveness of several fungicides to control rice blast" *Iranian Journal of Plant Pathology* 20 (1985): 35-45.

Khanzada MA, Rajput AQ and Shahzad S, "Effect of medium, temperature, light and inorganic fertilizers on *in vitro* growth and sporulation of *Lasiodiplodia theobromae* isolated from mango," *Pakistan Journal of Botany* 38 (2006): 885-889.

Kumara KLW, and Rawal RD, "Influence of carbon, nitrogen, temperature and P^H on the growth and sporulation of some Indian isolates of *Colletotrichum gloesporioides* causing anthracnose disease of papaya (*Carrica papaya* L.)," *Tropical Agriculture Research and Extension* (2008) 7-12.

Lee CL, Hung HK, Wang JJ and Pan TM, "Improving the ratio of monacolin K to citrinin production of *Monascus purpureus* NTU 568 under dioscorea medium through the mediation of pH value and ethanol addition," *Agriculture and Food Chemistry* 55 (2007): 6493-6502.

López, JLC, Pérez JAS, Sevilla JMF, Fernández FGA, Grima EM, Chisti Y, Production of lovastatin by *Aspergillus terreus*: effects of the C:N ratio and the principal

nutrients on growth and metabolite production. *Enzyme Microb. Technol.*, 33 (2003): 270-277.

Sangeetha CG, and Rawal RD, Nutritional studies of *Colletotrichum gloesporioides* (Penz.) Penz. and Sacc. The incitant of mango anthracnose. *World Journal of Agriculture Science* 4 (2008): 717-720.

Scardaci SC, Webster RK, Greer CA, e al , "Rice blast: A new disease in California. Agronomy Fact Sheet Series," Departement of Agronomy and Range Science, University of California, Davis. 1997-2, Available via DIALOG.
http://agronomy.ucdavis.edu/uccerice/AFS/agfs0297.htm, cited on 18 May 2000.

Tripathi SK, "Effect of media, pH, nitrogen, carbon sources and light on the growth and sporulation of *Pyricularia grisea*," *Annul of Plant Protection Science* 14 (2006): 166-168.