# Period, subculture number and culture color influence on the isolated *Magnaporthe oryzae* sporulation at the Tocantins State

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## Abstract

The fungus *Magnaporthe oryzae,* that caused the blast rice disease, present several restrictions for cultivation under laboratory conditions. Any characters can be variable even for the same isolate and need adaptations or innovations of the researchers for experimental development. This work aimed to verify the *M. oryzae* monosporic isolates of the IA-1 race collected at Projeto Rio Formoso, Formoso do Araguaia Municipality, the period of the cultures stored under laboratory conditions, the maximum quantity of the subcultures to keep the production of the conidia for the colonies satisfactory and the relation of different culture colors in BDA medium with the conidia production. For that, three experiments were carried out in complete randomized design. In the Experiment I, the cultures with 10, 14, 18, 22, 26 e 30 days after subculture were used. In the Experiment II, the cultures with 0, 1, 2, 3 and 4 subcultures from the original culture were used. In the Experiment III, the cultures were classified as Black, Center ash with wide white edges, Ash, Center ash with narrow white edges and White. Higher sporulation of the *M. oryzae* was obtained on the cultures with 14 days after the subculture, in the second subculture and on the black color.

**Key words:** *Pyricularia oryzae*; rice; blast disease; sporulation; phenotypic characters

## Introduction

Blast disease is one of the most important diseases known for the culture of rice (*Oryza sativa* L.) due to its wide geographic distribution and capacity to cause damage in plants (NUNES et al., 2007), huge damages in the grain productivity and quality (RANGEL et al., 1992). The agent which causes the rice blast disease is the fungus *Magnaporthe oryzae* (T.T. Hebert) M.E. Barr [anamorph *Pyricularia oryzae* (Cooke) Sacc.]. Even being a widely studied biological agent, *M. oryzae* presents a series of limitations of cultivation in controlled conditions.

Among the problems of the maintenance in vitro of isolates of *M. oryzae*, it is emphasized the crop aging, which normally causes loss of the vigor of conidiophores and reduction of the sporulation caused by the impoverishment of the mean of the culture and/or accumulation of metabolites excreted during the development of the microorganism in the substrate (APARECIDO et al., 2007). The necessity of periodic subculturing represents an exhaustive work. This practice results in problems as loss of the viability, morphologic and physiologic changes (aspect and color of the cultures), decrease and/or loss of the capacity of sporulation and, later, in most cases, in the loss of the pathogenicity of the isolates (BUENO et al., 2006; MEYER et al., 2006; VECHIATO et al., 2003; IGNOFFO et al., 1982).

In practice this changes which occur in the isolates come from the constant manipulation demanded due to the method of conservation which demand successive subculturing (APARECIDO et al., 2007). Therefore, the necessity of maintenance and manipulation of the isolates demand studies which characterize the effect of the preservation in vitro of the pathogenic and reproductive characteristics of the isolated of *M. oryzae*.

Facing these aspects, the work had as objective to evaluate the effect of successive subculturing

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over the viability and morphological characteristic of isolated of *M. oryzae* maintained in laboratory, collected in the Project Rio Formoso, municipality of Formoso do Araguaia, state of Tocantins.

## Material and methods

The work was performed in the Laboratory of Plant Pathology of the Universidade Federal do Tocantins – UFT (Federal University of Tocantins), Campus of Gurupi, state of Tocantins. It was performed three experiments – Experiment I: Effect of the age of the mycelium over the production of conidia of *M. oryzae*; Experiment II: effect of the number of subculturing over the production of conidia of *M. oryzae*; Experiment III: effect of the conservation in vitro over the color of the mycelium of *M. oryzae*. All the experiments were performed in the completely randomized design (CRD).

### Experiment I: Effect of the age of the mycelium over the production of conidia of *M. oryzae*

The experiment was installed with six treatments and four replications. It was used one isolate with one spore of *M. oryzae,* which belong to the breed IA-1, subculturing for Petri dished of 90 mm of diameter containing culture medium BDA 250 g potato, 20 g dextrose, 15 g agar and 250 mg ampicillin). The breed IA-1 was used because it was among the most prevalent in the state (DIAS NETO et al., 2008), was one of the most aggressive and has higher facility of sporulation. After subculturing the dished were conditioned in growth chambers (B.O.D.) under temperature of 25 ºC. Each plot was formed by two petri dished. The six treatments consisted on time (in days) of storage until the induction of the conidiogenous, which were: T10 – colonies with 10 days of storage; T14 – colonies with 14 days of storage; T18 – colonies with 18 days of storage; T22 – colonies with 22 days of storage; T26 – colonies with 26 days of storage; and T30 – colonies with 30 days of storage. In each treatment, for induction of conidiogenous, the petri dishes were maintained in acclimatized room with temperature between 25 and 28º C and air relative humidity of approximately 70%, remaining for 48 hours under continuous fluorescent light, covered with crepe cloth. For the evaluation, in each dish it was placed 20 mL of sterile distilled water and performed scrape with aid of a brush with softed bristles disconnect the conidia from the conidiophores. The suspension was filtered in gauze and the conidia quantified in Neubauer Chamber. Data were submitted to analysis of variance and averages compared by the Tukey test (p = 0.05) using the statistic program ASSISTAT (SILVA and AZEVEDO, 2006).

### Experiment II. Effect of the number of subculturing over the production of conidia *M. oryzae*

The experiment was constituted by five treatments and five replications. Each plot was formed by five petri dishes. It was used the following treatments: T0 – sporulation of the original culture, obtained from conidia collected from the leaf; T1 – sporulation obtained from the first subculturing of the original culture; T2 – sporulation obtained from the second subculturing; T3 – sporulation obtained from the third subculturing; T4 – sporulation obtained from the fourth subculturing. To obtain T0, one sample of leaf was taken from the refrigerator and cut only the fragments of the leaves with typical injuries from blast disease. These fragments, without asepsis, were placed in a petri dish with filter paper humidified with sterile water and taken to B.O.D. with temperature adjusted to 25 °C for 24 hours, aiming at enabling the sporulation of fungus in the injuries. With aid of a magnifying glass and a fine point needle, it was performed transference of the conidia from the sporulated injuries for five petri dished containing autoclaved culture medium agar-water (20 g agar for 1.0 L water). In each dish, it was placed conidia coming from a single injury. After the transference of the conidia, the dishes were sealed with PVC plastic, identified and placed in a B.O.D. with temperature adjusted to 25 °C for 48 hours. The dish which presented higher uniformity between germinated conidia was selected to be used in the experiment. With a stereoscopic microscope and with aid of a scalpel, five germinated conidia were transferred to dishes in medium BDA. The dishes were again incubated in B.O.D. in temperature

of 25ºC and, after twelve days in the dark, it was obtained the original culture (T0). To obtain T1, it was performed the subcutting from T0, for T2, from T1, and thus successively in intervals of 12 days. The induction of the conidiogenous, evaluation of the concentration of conidia and analysis of the data were performed with the same methodology described in the Experiment I.

### Experiment III. Production of conidia of isolates of *M. oryzae* grouped according to the color of the mycelium

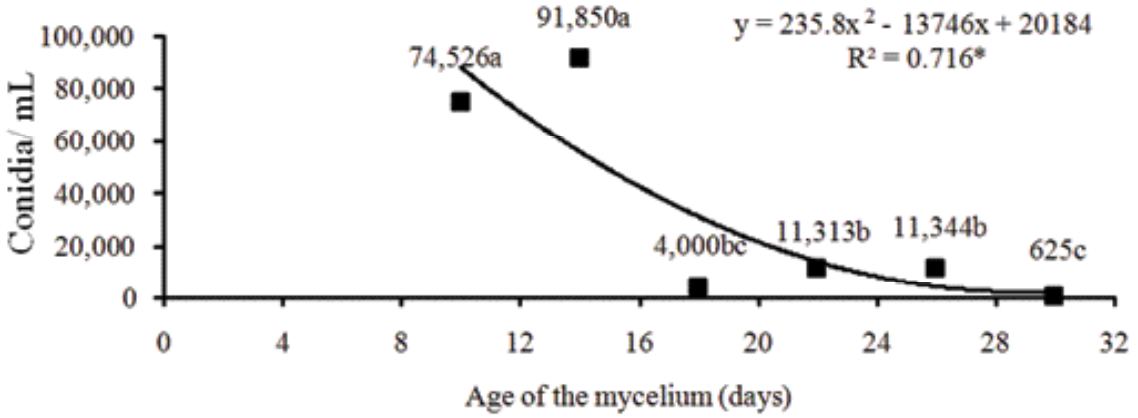
The experiment was conducted with five treatments and five replications. The treatments were the patterns of color of the mycelium of the isolates with one spore cultivated in BDA medium. It was used twenty five isolates with one spore grouped in five categories according to the color of the mycelium: Black (N), Middle gray with wide white edges (Ce), Grey (C), Middle gray with narrow white edges (Cl) and White (B). After grouping the isolates with one spore in their respective categories according to the typical color, then they were incubated in B.O.D. for 14 days in the dark, under temperature of 25ºC, when it was evaluated the color of the mycelium. For the induction of the sporulation, quantifying and statistical analysis of the data, it was used the methodologies previously described in experiment I.

## Results and discussion

### Experiment I: Effect of the age of the mycelium over the production of *M. oryzae*

The data obtained showed that the age of the mycelium had significant influence over the production of conidia in crops of *M. oryzae* (Figure 1). The best periods for sporulation occurred 10 and 14 days after subculturing, presenting, respectively, 74.526 x $10^3$ and 91.850 x $10^3$ conidia/mL. The most favorable period for the production of conidia occurred fourteen days after the subculturing. CRUZ and PRESTES (2007), evaluating the sporulation of *M. oryzae*, in medium BDA, obtained quantity similar to the mycelium with 10 days of age. PRABHU and FILIPPI (2006) determined that the best age for sporulation of the cultures of *M. oryzae*, in medium oak, was from eight to ten days.

After 14 days, the production of conidia dropped significantly, considering that at 18 days, the spore capacity decreased 95%. This fact may be related to the culture aging, which causes losses of vigor of the conidiophores and reduction of the sporulation due to the consumption of the medium of the culture and/or to the accumulation of metabolites excreted during the development of the microorganism in the substrate (APARECIDO et al., 2007).




*Averages follow by the same letter do not differ, according to the Tukey, at the level of 5% of probability. CV = 13.87% * $R^2$ significant at the level of 1%, with 22 degrees of release, according to LITTLE and HILLS (1978).*

**Figure 1.** Effect of the age of the mycelium over the production of conidia of *Magnaporthe oryzae*, which causes the rice blast disease maintained in petri dishes in BDA.

There was a small increase in the production of conidia in the period comprehended between 18 and 26 days, which may be related to the format ion of new sectors in the cultures. CASTRO et al. (2006), working with *Glomerella cingulata* f.sp. *phaseoli*, causer of anthracnose in common bean, observed formation of sectors after 30 days of culture development, with spontaneous formation of ascospores in these places. According to PRABHU and FILIPPI (2006), these sectors or saltations occur commonly and are formed during the establishment of isolated of one spore of *M. oryzae*. The same authors still comment that, the formations of this sectors are not very clear, however, they have been attributed in part to the dissociation of heterokariosis.

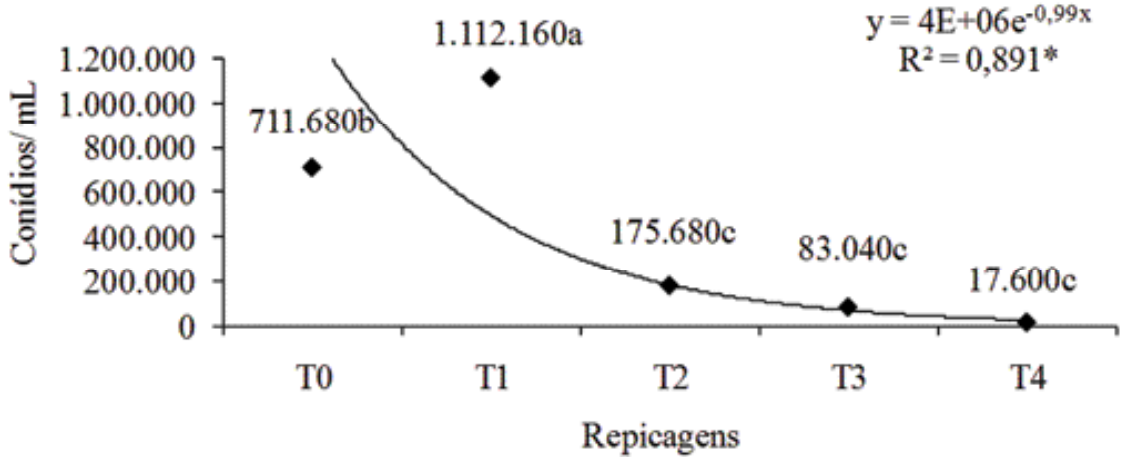
Another curious fact is that the sectors formed by a isolate with one spore, when being inoculated in rice plants, cause reactions of resistance and of susceptibility different from the main colony.

## Experiment II. Effect of the number of subculturing in the production of conidia *M. oryzae*

Successive subculturing in colonies of *M* . oryaze may cause changes in the quantity of conidia produced (Figure 2). The best conidiogenous were produced in the original culture (TO) and in the first subculturing (T1) of the crop, considering T1 superior to T0 in 400 x $10^3$ conidia/mL, with a significant statistic difference between them. The big sporulation which occurred in T1 may be related to the adaptation of the colony of the fungus to the culture medium.

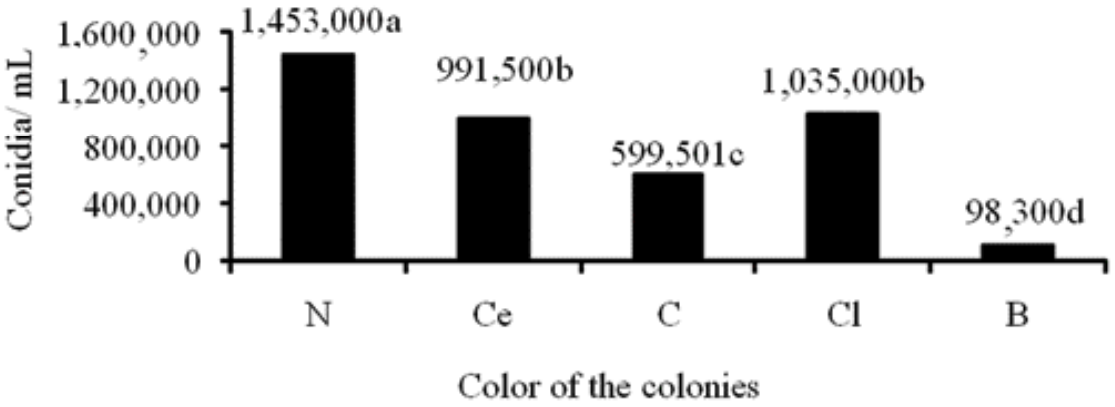
From the second subplanting (T2), there was a bid reduction in the capacity of sporulation in vitro of the cultures. Losses on the capacity of sporulation and infeccion of some pathogens are related by several authors. CAMARGO JUNIOR (2004), working with *Glomerella cingulata*, which cause anthracnose in common bean, verified that the exuberant production of perithecia in big clusters just happened in the first isolated dishes, and that successive subplanting of the initial isolation were losing the capacity of formation of reproductive structures either for parents as for cultures with one spore. VECHIATO et al. (2003), working with *Diaporthe* spp. and *Diaporthe phaseolorum,* which cause burn of the stem and the pod and the soybean stem canker, attributed the low virulence to the loss of pathogenicity due to procedures of successive subplanting during the period of maintaining the fungus in tubes containing BDA medium.

It must be also emphasized that, normally,




*Averages follow by the same letter do not differ, according to the Tukey, at the level of 5% of probability. CV = 13.87% * $R^2$ significant at the level of 1%, with 22 degrees of release, according to LITTLE and HILLS (1978).*

**Figure 2.** Effect of the number of subplanting over the production of conidia *Magnaporthe oryzae*, which causes the rice blast disease in petri dishes in culture medium BDA.




*Averages follow by the same letter do not differ, according to the Tukey, at the level of 5% of probability. CV = 16.39%*

**Figure 3.** Production of conidia of isolates of *Magnaporthe oryzae*, which causes rice blast disease grouped according to the color of the mycelium maintained in petri dished in BDA medium (N – Black; Ce – Middle gray with broad white edges; C – gray; Cl – Middle gray with wide white edges; and B – White).

samples maintained by period subplanting are stored in room temperature, and they are exposed to the alterations that it may occur. These alterations increase the frequency of mutations and, as a result, the culture may be lost (APARECIDO and FIGUEIREDO, 1997).

### Experiment III. Production of conidia of isolates of *M. oryzae* grouped according to the color of the mycelium

The production of conidia of *M. oryzae* was significantly different between the different patterns of color of the mycelium of the isolates with one spore (Figure 3). The highest production of conidia was obtained in the cultures with black color (N), followed by the cultures of Middle grey with wide white edges (Cl) together with Middle gray with narrow white edges (Ce). Cultures grouped in grey (C), even producing statistically less conidia than the previous, presented satisfactory sporulation. The lower sporulation was obtained in the color white (B). ANDRADE et al. (2007), studying the morphocultural and molecular characterization of isolates of *Colletotrichum gloeosporioides*, which cause anthracnose in papaya, grouped the cultures in different colors and verified that all the isolates were pathogens, independent on the color, presenting differences concerning the intensity of the virulence.

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