



## Research Article

# Effect of Rice Cercosporidium Caused by *Cercospora oryzae* Miyake on the Productivity of TS2 and IR841 under Controlled Conditions

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## Abstract

**Background and Objective:** In Benin, rice production has increased significantly over the past five years. Despite this increase, numerous parasitic constraints, particularly fungal, contribute significantly to the decline in productivity, making the country dependent on imports. The present study aimed at improving the knowledge of *Cercospora oryzae* to increase rice yields. **Materials and Methods:** In a controlled environment, a trial was set up using a split-Plot design. Agro-morphological parameters were evaluated based on observations and measurements according to the IRRI 2002 scale. **Results:** The results obtained reveal a negative impact of *Cercospora oryzae* on rice productivity. *Cercospora oryzae* hurts seedling emergence rates. In TS2, seedling emergence rates ranged from 65.10-98.85% for seeds occulted with *Cercospora oryzae* and 97.85% for um inoculated seeds. Cercospora symptoms were observed on the leaves and stems of all plants except those not inoculated with varying degrees of severity. **Conclusion:** The average number of productive tillers decreased when inoculated early. Inoculation at the swelling and heading stages reduced the weight of 1000 grains. Cercosporidium negatively impacts rice growth and yield.

**Key words:** Rice, *Cercospora oryzae*, disease, yield, degrees of severity

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Rice constitutes one of the main self-consumption crops in Benin. Like cassava, maize, banana and palm oil, it is an important source of household income<sup>1</sup>. In recent years, although there has been an increase in global rice production in general<sup>2</sup>, it is subject to numerous biotic constraints, including fungal diseases, which contribute significantly to the decline in yields in the various production systems<sup>3</sup>. These parasitic constraints can nevertheless be exerted by pathogens of various origins<sup>4</sup>. In the case of rice production, the most important threats are caused by fungi *Pyricularia oryzae*, *Helminthosporium oryzae*, *Curvularia lunata*<sup>5</sup> but also *Cercospora oryzae*, which is responsible for characteristic leaf spots and is one of the main limiting factors of rice cultivation in Benin<sup>6</sup>. Cercosporiose is present in most production areas. Its distribution is not yet confirmed on a global scale<sup>7</sup>. The four main fungal pathogens of rice are mainly distinguished by their morphological characteristics: Conidia and conidio spores. The narrow brown spot disease or cercosporiosis caused by *C. oryzae* is more recent in appearance<sup>6</sup> than blast or helminthosporiosis. *Pyricularia oryzae* is considered the most destructive of the leaf disease complex<sup>4</sup>. Cercosporidium is also widely distributed throughout the world, with much more rapid development on susceptible cultivars and a much wider host range<sup>7</sup>. Rectilinear lesions are often observed on 5-6 weeks old rice leaves and the economic consequences are severe as losses can represent 55% of the crop<sup>8</sup>. Very little work has been done on cercosporiose in Benin. More recently, a study was conducted on the incidence and distribution of fungal diseases of rice in southern Benin. The latter study revealed that cercosporiose of rice is among the main phytosanitary problems affecting rice fields in Benin, along with blast and helminthosporiosis<sup>9</sup>. The present study focused on the manifestations of *Cercospora oryzae*, including periods of occurrence, level of aggressiveness and effects on productivity under controlled conditions. The rice development sector in West Africa requires research on so-called minor diseases. The rice development sector in West Africa requires research on so-called minor diseases, as all diseases that can lead to yield reductions, even if minimal, must be taken into account to optimize rice production.

## MATERIALS AND METHODS

**Study site:** The trials were carried out in the Laboratory of Plant Physiology and Environmental Stress Studies (Plant Pathology and Plant Protection Unit) at the University of Abomey-Calavi, Faculty of Sciences and Techniques in 2019.

**Plant material:** Two rice varieties were used in the study. The intraspecific variety TS2 of the species *Oryza sativa* and the interspecific variety IR841, resulting from a cross between *Oryza glaberrima* Steud. and *Oryza sativa* L. *Cercospora oryzae* was isolated from infected rice leaves from the Docomey rice plain<sup>4</sup>. The fungus was regenerated on a rice flour culture medium in the laboratory for 21 days with 12/12 hrs light-dark alternation.

**Preparation of culture media:** Rice flour culture medium was prepared for 1000 mL of distilled water by mixing 20 g of rice flour with 4 g of yeast extract added to 15 g of agar-agar. The mixture was autoclaved under a pressure of 130 bars at a temperature of 121 °C for 15 min and poured into sterilized 9 cm diameter Petri dishes at a rate of 15 mL per Petri dish.

**Experimental device:** The experimental setup use dishes split-plot with 32 treatments in 3 replications and each treatment has 02 plants.

Test consists of three factors:

- The variety factor (V) includes two levels: The interspecific variety Sativa × Glaberrima IR841 (V1) and the intraspecific variety TS2 (V2)
- The fertilizer type factor (V) has four levels:
  - **F0:** No manure application
  - **F1:** Application of NPK complex fertilizer as a bottom dressing
  - **F2:** Urea
  - **F3:** Organic manure (dried cow dung)
- The plant inoculation stage factor (S) consists of four levels
  - **S0:** Non-inoculated control (seed soaking in sterile water)
  - **S1:** Inoculation of grains before sowing
  - **S2:** Inoculation at swelling stage (80 days sowing)
  - **S3:** Inoculation 10 days after rice heading

**Conduct of the trial:** Seedling is done with rice grains that have been disinfected on the surface by soaking in a 1% solution of sodium hypochlorite (NaOCl) for 2 min. These grains are then shaken for 5 min, rinsed with sterile distilled water and wrung out with sterile blotting paper<sup>10</sup>. Four seeds were sown in plastic pots with a bottom diameter of 23.5 cm, an opening diameter of 23.5 cm and a depth of 23.5 cm. Ade-mating with two plants per pot was carried out on the 10th day after sowing (DAS). Fertilization consisted of a basic fertilizer application as follows: 1.3 g of NPK (14-23-14) per pot,

equivalent to a dose of 300 kg ha<sup>-1</sup> and 0.9 g of urea/pot, equivalent to a dose of 150 kg ha<sup>-1</sup>. For the organic manure treatment, dried cow dung is mixed with potting soil in the proportion of about 3% of the soil weight. The soil used came from the University of Abomey-Calavi garden and was sterilized in a mechanical autoclave at 121 °C for 30 min before being put into plastic pots.

Two inoculation techniques were used in the work:

- Seed inoculation is performed by depositing rice grains on a 21 days old mycelia colony of the fungus for 24 hrs. These grains are then incubated for 48 hrs at an average temperature of 28 °C, they are removed and sown immediately in the pots<sup>10</sup>
- Inoculation of plants at the swelling and heading stages is carried out by foliar spraying at 10<sup>7</sup> spores mL<sup>-1</sup>. For this purpose, plants in each pot receive approximately 10 mL of spore suspension at 37 °C. The plants are sprayed under a plastic sheet. After spraying, high humidity is created and maintained for ten days, to favour infection.

**Parameters assessed:** Observations were made on the following variables:

- The rate of seedling emergence
- Leaf severity
- The number of productive tillers per plant
- Weight of a thousand grain

**Statistical analysis:** The data collected during the experiment were entered using Excel and subjected to statistical analysis with R software. An Analysis of Variance (ANOVA) and a separation of means using the Student-Newman-Keuls test was performed at the 5% probability level.

## RESULTS

### Effect of *Cercospora oryzae* on seedling emergence rate:

Data on the effect of *Cercospora oryzae* inoculation on rice seedling emergence are shown in Table 1. The emergence rates ranged from 65.10-74.17% for seeds inoculated at sowing (S1) and from 97.48-98.52% for non-inoculated seeds (S0, S2, S3). The results obtained indicate a reduction in seed germination under the effect of inoculum of 24.35% for IR841 and 33.20% for TS2. Statistical analysis of the emergence rate of seedlings at 7 days after inoculation showed a significant difference only between the different inoculation stages. Thus, the emergence rate obtained at S1 (seed inoculation at

Table 1: Seedling emergence rates according to inoculation stages 7 DAS

Varieties	Inoculation stage (*)	Average percentage of emerged panicles
IR841	S0	98.27 <sup>a</sup>
	S1	74.17 <sup>b</sup>
	S2	97.48 <sup>a</sup>
	S3	98.52 <sup>a</sup>
TS2	S0	97.85 <sup>a</sup>
	S1	65.10 <sup>b</sup>
	S2	98.85 <sup>a</sup>
	S3	98.30 <sup>a</sup>
F <sub>cal</sub>		5.992
P		0.0009
Level of significance		HS

Numbers with the same letter in the column are statistically equivalent according to the SNK test at the 5% threshold, S0: Non-inoculated control (seed soaking in sterile water), S1: Inoculation of grains before sowing, S2: Inoculation at swelling stage (80 days sowing), S3: inoculation 10 days after rice heading, HS: Highly significant difference, P: Probability, F<sub>cal</sub>: Calculated frequency, DAS: Days after sowing

sowing), is statistically different from the other inoculation stage and the non-inoculated control. The other treatments studied (S0, S2 and S3) did not differ from each other.

### Assessment of the severity of leaf attack due to *Cercospora oryzae* according to the stages of inoculation of the plants:

The results for leaf severity of cercosporidium showed in Fig. 1. The disease was absent on non-inoculated plants (S0). When plants were inoculated at the swelling stage (S2), symptoms appeared after 7 days (DAI), with a severity score ranging from 1.25 (V1F1S2) to 2.63 (V2F0S2). After 21 days, the score reached 4.50 (V2F0S2). The severity score was 1.75 for V1 F0S2 and 1.63 for V1 F3S2 7 days after inoculation at the swelling stage. They were 2.63 and 1.50 for V2 F0S2 and V2 F3S2, respectively. This trend is observed up to 28 DAI. The variety TS2 (V2) was more susceptible to infection than IR841 (V1). Inoculation of plants at the heading stage (S3) also caused symptoms after 7 days. The attack severity Scores ranged from 1.88 (V1F0S3) to 3.13 (V2F3S3). The effect of fertilization was less visible at this level. The hybrid variety TS2 (V2) was more susceptible than IR841 (V1), with a maximum score of 3.13 and 2.75, respectively. Infection from inoculation at the rice swelling stage (S2) slowed down, with a maximum score of 3.50 (V2F0S2). Analysis of variance of the leaf attack data indicates a significant difference related to inoculation as early as day 7 after inoculation of the plants. Differential response of varieties was observed two weeks after the inoculation of plants at the rice swelling stage. This interaction is observed after 7 days when inoculation is done at the early heading stage. At the swelling and heading stages, statistical analysis revealed a higher susceptibility of the TS2 variety to *Cercospora oryzae* leaf attack.

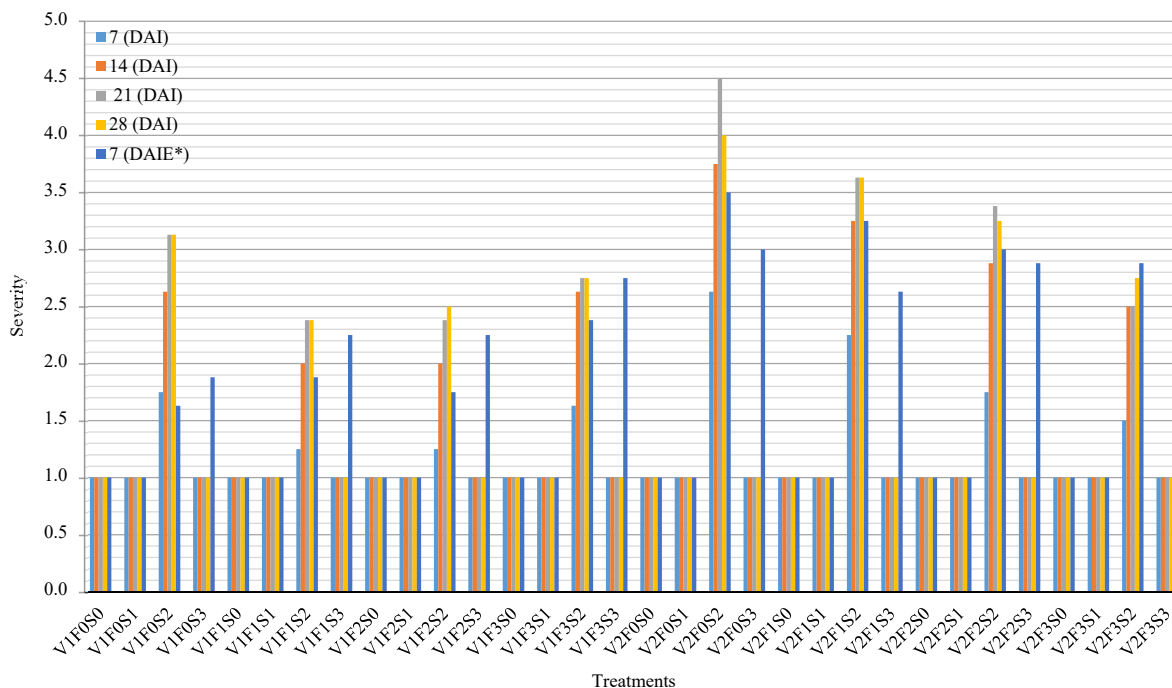


Fig. 1: Average severity of leaf attack after inoculation at the swelling and heading stage of rice

S0: Non-inoculated control (seed soaking in sterile water), S1: Inoculation of grains before sowing, S2: Inoculation at swelling stage (80 days sowing), S3: Inoculation 10 days after rice heading, V1: IR841, V2: TS2, F0: No manure application, F1: Application of NPK complex fertilizer as a bottom dressing, F2: Urea, F3: Organic manure (dried cow dung)

Table 2: Tall age and productivity of rice according to inoculation stages

Stage of inoculation	Productive tillers	Infected panicles (%)	Infected seeds (%)
S0	14.28 <sup>b</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>
S1	06.71 <sup>a</sup>	32.06 <sup>b</sup>	02.76 <sup>b</sup>
S2	11.59 <sup>a</sup>	28.30 <sup>a</sup>	02.98 <sup>b</sup>
S3	12.64 <sup>a</sup>	26.24 <sup>b</sup>	04.04 <sup>a</sup>
F <sub>cal</sub>	6.716	93.078	02.857
P	<0.001	<0.001	<0.001
Level of significance	HS	HS	HS

Numbers with the same letter in the column are statistically equivalent according to the SNK test at the 5% threshold, HS: Highly significant difference, P: Probability, F<sub>cal</sub>: Calculated frequency

**Impact of inoculation on tillering, rice productivity and grain quality:** Data on the number of productive tillers, the rate of infected panicle sand grains and the weight of 1000 grains are grouped in Table 2.

**Number of productive tillers:** The average number of productive tillers ranged from 14.28 for non-inoculated plants (S0) to 11.59 for plants inoculated at the swelling stage (S2). This value is 6.71 for inoculated seed plants and 12.64 for inoculated plants at the heading stage. Inoculation significantly ( $p < 0.001$ ) reduced the number of productive tillers compared to non-inoculated plants (S0).

**Rate of infected panicles:** The results of the assessment of the rate of panicles with narrow brown spots parallel to the Midrib are shown in Table 2. *Cercosporidium* was present on all

plants except those that were not inoculated. The analysis of variance and the Student-Newman-Keuls (SNK) test for separation of means indicated a highly significant difference ( $p < 0.0001$ ) between the means of the infection rates of the panicles. Infection from seed and plant inoculation at the swelling stage was transmitted to the panicles.

**Percentage of damaged grains per panicle:** Data on the number of infected grains are grouped in Table 2. Statistical analysis of the data on the rate of grains with narrow brown spots revealed that plants inoculated at the heading stage differed statistically from the others ( $p < 0.001$ ), with an average rate of 4.04% of spotted grains compared to 2.76% for plants from inoculated seeds (S1) and 2.98% for plants inoculated at the swelling stage (S2). Inoculation of rice at the swelling stage resulted in severe grain spoilage.

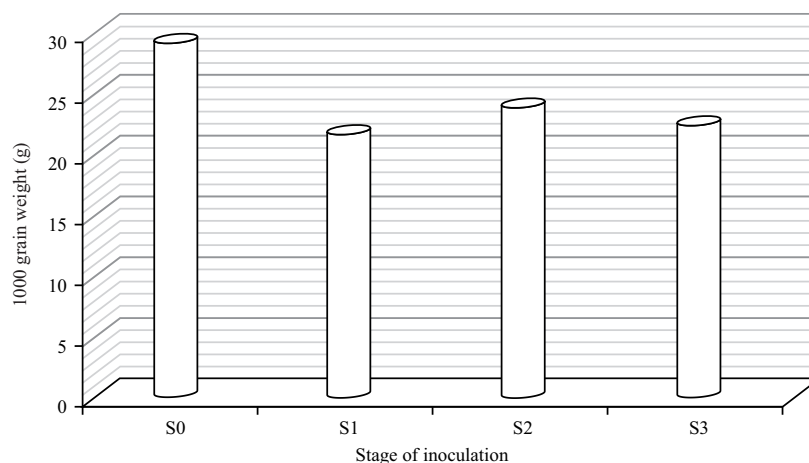


Fig. 2: Weight of 1000 grains depending on the inoculation stages

S0: Non-inoculated control (seed soaking in sterile water), S1: Inoculation of grains before sowing, S2: Inoculation at swelling stage (80 days sowing), S3: Inoculation 10 days after rice heading

Table 3: Results of the regression analysis of the average weight of 1000 grains on these varieties of leaf attack

Parameters	Observation dates in days after inoculation (DAI)				
	7 DAI (S2)	17 DAI (S2)	21 DAI (S2)	28 DAI (S2)	7 DAI (S3)
Value of R	0.390	0.408	0.415	0.415	0.450
R2 value	0.152	0.166	0.173	0.172	0.203
F <sub>cal</sub>	5.376	5.991	6.529	6.242	7.618
P	0.027	0.020	0.018	0.018	0.010
Meaning	S	S	S	S	HS

S: Significant difference, HS: Highly significant difference

**Weight of 1000 grains:** The average 1000 grain weights at 14% moisture content are shown in Fig. 2. Grains inoculated before sowing (S1) and heading (S3) stages had the lowest average 1000 grains weights of 21.63 and 22.37 g, respectively. Inoculation before sowing and heading stage reduced the 1000 grains weight by 7.5% in S1 and 6.76% in S3 compared to non-inoculated plants S0. The analysis of variance and the comparison of means test (SNK) showed significant differences related to the factors varieties ( $p < 0.0001$ ), inoculation ( $p < 0.0001$ ) and manure ( $p = 0.002$ ). There was a significant interaction for the variety by manure effect ( $p < 0.0001$ ). There was also a highly significant difference between the different stages of inoculation ( $p < 0.0001$ ). The response of rice to the action of the fungus would thus be related to the stage of infection, variety and soil fertility.

**Relationship between the incidence of cercosporidium leaf blight, rice productivity and transmission of infection to grain:** An analysis of the relationship between these varieties of the disease according to the stages of inoculation on the one hand and the productivity of the rice (weight of 1000 grains,

weight of the panicle), the rate of healthy panicles and grains, on the other hand, made it possible to establish the regression and correlation coefficients. The results of this analysis are shown in Table 3 and 4. The correlation coefficient between leaf incidence and 1000 grain weight varied from -0.390 to -0.450 during the observation period. The correlation coefficient between germination rate and other variables ranged from -0.295 to 0.389. The healthy panicle rate, average grain weight per panicle and healthy grain rate seem to be mainly dependent on the incidence of leaf attack at the heading stage (7 DAE), with a correlation coefficient of 0.561, -0.348 and 0.508, respectively. The results of the analysis show a significant relationship between leaf disease incidence and 1000-grain weight. The relationship between foliar disease incidence and 1000-grain weight (P1000 grains) is stronger the later the infection occurs. For the value of incidence observed 7 days after inoculation of rice at the heading stage, this coefficient is 0.45 ( $P = 0.01$ ) and the coefficient of determination ( $R^2$ ) is 0.20. Foliar disease incidence at the heading stage seems to have the strongest influence on rice productivity under the conditions of the current study.

Table 4: Matrix of correlation coefficients between the variables studied

Variables	Germ (%)	SFM7	SFM14	SFM21	SFM28	SFM7JAE	PanS	GrainS	P1000grs	Pgrains
Germ (%)	1.000	-	-	-	-	-	-	-	-	-
SFM7	0.371	1.000	-	-	-	-	-	-	-	-
SFM14	0.340	0.983	1.000	-	-	-	-	-	-	-
SFM21	0.353	0.986	0.996	1.000	-	-	-	-	-	-
SFM28	0.354	0.985	0.998	0.997	1.000	-	-	-	-	-
SFM7JAE	0.389	0.572	0.577	0.580	0.575	1.000	-	-	-	-
PanS	0.072	0.308	-0.315	-0.313	-0.317	0.561	1.000	-	-	-
GrainS	0.019	-0.292	-0.299	-0.297	-0.301	0.508	0.954	1.000	-	-
P1000grs	-0.295	-0.390	-0.408	-0.415	-0.415	-0.450	-0.092	-0.082	1.000	-
Pgrains	-0.153	-0.072	-0.073	-0.071	-0.071	-0.348	-0.381	-0.351	0.477	1.000

Germ%: germination rate, SFM7: Diseased leaf surface 7 days after inoculation at the swelling stage (S2), SFM14: Diseased leaf surface 14 days after inoculation at the swelling stage (S2), SFM21: Diseased leaf surface 21 days after inoculation at the swelling stage (S2), SFM28: Diseased leaf surface 28 days after inoculation at the swelling stage (S2), SFM7JAE: Diseased leaf surface 7 days after inoculation at the heading stage (S3), PanS: Healthy panicles (%), GrainS: Healthy grains (%), Pgrain: Average grain weight per panicle, P1000grs: 1000 grains weight

## DISCUSSION

The fieldwork has shown the negative impact of Cercosporiose on rice productivity. This impact is reflected in the reduction of grain quantity and quality. Current results are similar to those previous studies<sup>10,11</sup>, who showed that rice blast had the same impacts. It was found that when grains were inoculated with the spore suspension of  $10^7$  spores mL<sup>-1</sup>, the germination rate was reduced compared to untreated grains. This lack of germination, which can reach 40%<sup>12</sup>, has as a main consequence a lower density of plants in the field. The infection resulting from the inoculation of plants at the swelling stage of rice continues after heading. At this stage of rice development, observations revealed variety-related effects but not manure-related effects. This can result in greater or lesser yield losses if reseeded is not carried out. These results are similar to those obtained previously<sup>9</sup> in a field setting where inoculation resulted in a significant reduction in germination rate of about 39 and 32% for the varieties TS2 and IR841, respectively. Seed-borne fungi are very diverse in nature<sup>13</sup>. Seedlings from inoculated grains experienced stunted growth, which was reflected in reduced height during the early stages of growth. Similar observations were made<sup>10,14</sup> who also noted that plants from infected seeds were smaller in size. This delay in growth could be explained by the inhibition of root growth by the toxins produced by the fungus<sup>15</sup>. When inoculated to seeds, the fungus also produced a decrease in the number of tillers. The number of productive tillers is also reduced by the fungus. The lack of fertilization favours the development of the disease, as indicated by the results of the statistical analysis. Cercosporiosis can be considered as the "poor farmer's disease", which is characterized by poor soil fertilizer agriculture. The lack of elements such as phosphorus, nitrogen and potassium favours the attacks of *Cercospora oryzae*. The impact of the disease on

plant productivity is greater when infection occurs at the heading stage. This is probably due to the reduction in photosynthetic efficiency, following the development of lesions on the panicle leaf<sup>14</sup>. There was a significant correlation between leaf incidence at the heading stage of rice and grain weight per panicle. These results confirm the work on the behaviour of varieties in the field, which showed that the full tillering, bolting and early heading stages is when rice is most susceptible to *Cercospora oryzae* disease. From this observation, it is possible to choose the best time for estimation of potential losses based on the foliar incidence of cercosporiose. Finally, the study shows that the variety TS2 has a higher susceptibility to foliar attack at the bolting and heading stages.

## CONCLUSION

The results obtained during the study on the problem of cercosporiosis in rice confirm the importance of this fungal disease in Benin through its impact on rice productivity under controlled conditions. Cercosporiosis harms rice productivity. This is reflected in reduced germination, delayed plant growth, reduced tillering and, above all, reduced yield due to poor grain filling. The cercosporiosis study revealed a reduction in germination ranging from 37-58%. Seeds inoculated at the seed stage experienced a delay in growth which was reflected in the reduction in height during the early stages of growth (21 DAS, 28 DAS). In addition, the fungus inoculated to the seed resulted in a decrease in tillering. The number of productive tillers is also reduced by the fungus. The primary inoculum carried by the seed has negative effects over time. Current results confirm the predominant role of infection at the advanced stages of rice development in the transmission of the fungus to the seed, these are the swelling and heading stages. It is necessary to disinfect the seed before planting to

avoid the use of pre-contaminated semen. The results obtained highlight the importance of balanced fertilization of rice in the expression of resistance to cercosporiosis in rice. Cercosporiosis is a serious potential threat to the country's growing rice production.

### SIGNIFICANCE STATEMENT

This study highlighted the impact that cercosporidium can have on rice production. It will help producers improve their yields but also it opens a door for researchers to create varieties with horizontal resistance.

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