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Study of climatic factors on the population dynamics of *Pyricularia oryzae* on some varieties of paddy rice (*Oryza sativa*)

SOPIALENA[1,♥], PRATIWI JATI PALUPI[2,♥♥]

[1]Program of Plant Pests and Diseases Science, Faculty of Agriculture, Universitas Mulawarman. Jl. Pasir Balengkong, Kampus Gunung Kelua, Samarinda 75119, East Kalimantan, Indonesia. Tel: +62-541-749161, Fax: +62-541-738341. ♥email: sopialena88@gmail.com

[2]Program of Agricultural Industrial Technology, Faculty of Agriculture, Universitas Nahdlatul Ulama. Jl. KH. Harun Nafsi Gg. Dharma, Rapak Dalam, Loa Janan Ilir, Samarinda 75251, East Kalimantan, Indonesia. Tel./Fax.: +62-541-7269413, ♥♥email: pratiwi.jp@gmail.com

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Abstract. *Sopialena, Palupi PJ. 2017. Study of climatic factors on the population dynamics of Pyricularia oryzae on some varieties of paddy rice (Oryza sativa). Biodiversitas 18: 701-708.* The occurrence of plant diseases is influenced by three factors: pathogen, environment, and host. Environment playing important role in the development of the disease is the climatic factor, whereas the resistance of plants also influences the development of disease. The research was conducted during five months from April to August 2016 and the location of the research was in paddy fields in Tanah Merah, North Samarinda, East Kalimantan, Indonesia. The study aims to determine the climatic factors (temperature and humidity) which are most dominant to the rate of spot width, the infection rate of pathogen attack intensity on *Pyricularia oryzae* and the development of *P. oryzae* spores in several varieties of paddy rice (Inpari 7, Ciherang and Cibogo). The parameters in the study are climate factors namely humidity and temperature, the width rate of spot blast disease, the attack intensity of pathogenic *P. oryzae* and the spores number of *P. oryzae*. Research shows that the population of *P. oryzae* is strongly influenced by temperature. Low temperature is the most dominant factor affecting the rate of infection of blast disease. The number of pathogen population also affects the attack intensity of the disease. A higher population of *P. oryzae* led to higher intensity of blast disease in rice plants. Moreover, varieties of rice plants also affect *P. oryzae* attack. The more vulnerable the plants, the higher level of *P. oryzae* attack on the plant. The lowest intensity attack of the disease is found in Ciherang, then Cibogo and the most vulnerable is Inpari 7.

Keywords: Climatic, *Oryza sativa*, *Pyricularia oryzae*

INTRODUCTION

The rice plant (*Oryza sativa* L.) as a producer of rice is one of the main food commodities and it plays an important role in the fulfillment of basic needs for the people of Indonesia. Efforts to increase rice production face many obstacles and one of them is plant pathogens. One disease frequently attacking the rice plant is blast disease. Blast disease is caused by *Pyricularia oryzae* fungi belonging to the Ascomycetes. The morphology of *P. oryzae* is to have round, oval, opaque conidia with two insulations (3 rooms). One cycle of blast disease begins when the spores start to infect and produce a spot on the rice plant and it ends when the fungi begin sporulation and deploy new spores through the air. In a suitable environment condition, one cycle of blast disease can occur in about one week, and one spot can produce spores for more of twenty days. In suitable humidity and temperature, *P. oryzae* can experience many cycles and produces an abundance of spores at the end of the season. This high level of inoculum is very harmful to the vulnerable rice plants (Mentlak et al. 2012).

Blast disease is cosmopolitan meaning that it attacks rice plants throughout the world. In Indonesia, the attack of blast disease (*P. oryzae*) can reach an area of 1,285 million ha or 12% of total rice field areas in Indonesia (Kharisma et al. 2013). This fungus appears since there are varieties that are susceptible to the pathogen, and it is also influenced by environmental factors and the way of farming that uses too much nitrogen fertilizers as well as high rainfall and humidity (Hajano et al. 2013). This disease can damage all parts of the plant and can attack at all phases of the rice plant. The high intensity of the blast pathogen attack of *P. oryzae* height can be seen in the young rice crops. The resistance of rice plants to *P. oryzae* increases as they grow older. This is supported by the opinion of Kahn and Libby (1958) and Suriani et al. (2015) that the leaf susceptibility to the infection of blast disease declines along with the increasing of the age of rice plants, so the number of spotted sporulated leaves will decrease. The ability of spot to form conidia varies depending on the shape and size of the spot (Hayashi and Yoshida 2009; Yuliani and Maryana 2014).

Economically, the attack of blast disease in the nursery was not as severe as the attack of rotten neck on mature plants. The heavy attack in the nursery or seedling stage could cause the plants dried up, while heavy attacks in adult plants causing rotten neck can result in harvest failure and the unavailability of seeds for the following planting season (Yuliani and Maryana 2014). The high losses caused by blast disease have led to the continuity in the development of high-yielding varieties that are resistant to blast disease, but the instability of rice varieties to blast disease causes pathogens to easily break the resistance of varieties, especially when the resistance is determined by a dominant gene.

The disease is a process engaging various elements such as climate factors. In the field, against the resistance of rice varieties, the behavior of disease is often associated with climatic factors, so the implementation of a good control of plant diseases needs a good knowledge of climatic factors. Assessment of factors affecting the pathogen population is expected to assist in determining the breeding assembly and deployment strategy of new varieties (Mc Donald 1997; McDonald 2004; Klaubauf 2014). In addition, the study on blast fungus population dynamic in the field is the first step that must be done to get information about a plant with resistive genes which is effective for a particular location (George et al. 1998). It is necessary for a study of the climatic factors on the population dynamics of *P. oryzae* on some rice varieties (*Oryza sativa*) in the city of Samarinda and Kutai Barat regency. The study aims to determine the most dominant climatic factors (temperature and humidity) on the rate of spot width, the rate of infection intensity of the pathogen *P. oryzae* attack and the number of *P. oryzae* spores on paddy varieties (Inpari 7, Ciherang and Cibogo) and to determine the effect of the number of *P. oryzae* spores on the attack intensity of pathogen *P. oryzae* toward paddy varieties (Inpari 7, Ciherang and Cibogo).

MATERIALS AND METHODS

The research was conducted for five months from April to August 2016. The research location was in paddy fields of Tanah Merah in North Samarinda, East Kalimantan, Indonesia, while laboratory research was conducted in the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia.

Research design

In the field, the research was done by observing the climatic factors (humidity and temperature) on the progression of the width of spot blast disease, the intensity of the blast disease attack and the number of *P. oryzae* spores. The observation was carried out on rice crops in three different fields. Eight samples were taken from each field, and then were averaged to yield observation results. In the laboratory, the research was done by observing the biology of *P. oryzae* (the development of the colony on the emergence and development of conidia)

Observations of temperature and humidity on the field

Temperature and humidity are climatic factors that have an important role in the life of the organism, namely, in this study, the plants and the pathogens causing disease (Hajano et al. 2013; Ghini et al. 2008). Observations of temperature and humidity were done using a digital thermometer and HTC-1 hygrometer. Thermometer and hygrometer were placed above the rice fields with a height of 50 cm. The tools were left for 1 minute in order to obtain a stable range of temperature and humidity and then the results were recorded. The observation was done daily at 06.00 WITA.

Table 1. Temperature and humidity

Observation on weeks of	Temperature (°C)	Humidity (%)
2	35.6	69
3	29.6	75
4	28.7	78
5	29.4	85
6	28.3	86
7	29.8	86
8	30.3	85
9	25.8	80
10	24.2	81
11	22.7	88
12	22.3	97

Observation on the number of spores in the field

Observation on the number of spores in the field was done using an object glass that has been coated by double cellophane tape on its two sides and was put on the leaves of rice plants at the height of 20-30 cm from the ground. The glass object was placed in the afternoon and was taken back the next morning. The number of spores found on the object glass was observed with a light microscope. The spore observations were carried 6 times; 3 times during the vegetative phase (age of 7, 14 and 21 days after planting) and 3 times during the generative phase (age of 45, 65 and 90 days after planting).

Observation of the intensity of blast disease attack on leaves in rice field and the rate of disease infection

Observation of the intensity of the blast disease attack (*Pyricularia oryzae*) was carried out on week 1 after planting in the field and the observation was done every week. The observations were carried out 12 times. The intensity of the disease attack can be calculated by the following formula (Biogen BB 2007).

$$I = \frac{\Sigma(nxv)}{N\ X\ Z}\ x\ 100\%$$

Where:

I : Intensity of the disease attack (%)
n : Number of attacked leaves
v : The value scale of the attacked leaves
N : The total number of observed leaves
Z : Highest Scale of attack scale category

Table 2. Attack scale category on the leaves according to Biogen BB (2007)

Scale	Attack category	Description
1	1-5% attack on leaf width	Resistant
3	5-11% attack on leaf width	Light
5	> 11-≤ 25% attack on leaf width	Medium
7	> 25-≤ 75% attack on leaf width	Heavy
9	> 75-100% attack on leaf width	Parched

Calculation of the rate of disease infection can be calculated using the formula:

$$r = \frac{2{,}3}{t} \log \frac{x_t}{x_0}$$

Where:
r : Rate of infection
2.3 : Conversion result of natural logarithms to normal logarithms (lnx : 2.3Logx)
t : Observation time lapse
x_t : The proportion of infected leaves at t time
x_0 : Proportion of infected leaves at the beginning of observation

The making of PDA media

The media of Potato Dextrose Agar (PDA) is made of potatoes with a composition of 250 g of potatoes, 1000 ml of distilled water, 20 g of gelatin stick, and 20 g of powdered sugar. The steps are: peel the potatoes, cut into small dice, add them with 500 ml of distilled water and put on the stove until the potatoes were softened, and then remove them from heat and strain them and set them aside. Next, mix 500 ml of distilled water with 20 g of gelatin sticks and cook until gelatin were dissolved. Put the first liquid (potato-boiled water) into the solution, and then add with distilled water to reach 1000 ml in volume and stir them at the same time, and cook the mixture for a few minutes. Pour the media into sterilized Erlenmeyer tube and cover it with cotton and aluminum foil. Sterilize it in an autoclave at 120°C with a pressure of 2 atm for 20-30 minutes. Once sterilized, the medium was transferred from the tube into a Petri dish aseptically to keep it from contamination.

Isolation of *Pyricularia oryzae*

Before performing insulation, sterilize the Enkas with alcohol 90% and also sterilize the tools in sterilizer oven and sprayed them with alcohol 70% at the time of use. Cut the parts containing spot blast, sterilize them with alcohol 70% and rinse them again with sterile distilled water, and set aside. Pour the PDA media into petri dish over a Bunsen flame, and let it stand for 15 minutes to be cooled and hardened. Once the media has hardened, isolate the spot blast piece that was sterilized earlier in the media and divide it into three dots then plaster them tightly and labeled. This insulation activity was carried out over the bunsen fire to keep it sterile.

Observation on the biology of *Pyricularia oryzae*

This activity includes the observation on the development of colonies of *P. oryzae*, the emergence of *P. oryzae* conidia and its development for nine days at every 10:00 am WITA at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia.

Variable response

Data retrieval

The primary data retrieval is done on each area in the rice field and the laboratory. Primary data collected are: the size of the spot of blast disease on the leaves in the rice field; the attack intensity of blast disease on the leaves in the rice field; the number of *P. oryzae* conidia in the rice field; the temperatures in the rice field; the humidity in the rice field; the soil nutrients in the rice field (N, P and K); the development of colonies of *P. oryzae* in the laboratory; the time of occurrence of *P. oryzae* conidia in the laboratory; *P. oryzae* conidia development in the laboratory.

Data analysis

The study used linear regression analysis and multiple linear regression and the steps to verify the research results were the F test at 5% level, and the t-test at 5% level.

RESULTS AND DISCUSSION

The progression of *Pyricularia oryzae* pathogen attack intensity (%)

Based on the research result, the progression of *P. oryzae* pathogen attack intensity on Inpari 7, Ciherang and Cibogo varieties for 12 weeks showed that the Ciherang still has resistance against the attack of *P. oryzae* pathogen (Table 3), although these types of rice were from the hairless group and with low production of saplings where spores are more easily sporulated on the leaf surface, but with good spacing, they can have good resistance to blast disease. Plant disease arises because of the influence of the environment and cultivation practice in the form of a tiny spot on the leaves which gradually becomes large and in the shape of a parallelogram (Figure 2).

The effect of temperature on the rate of blast disease infection

Plant disease arises because of the susceptible varieties toward pathogen and sensitive to temperature. In Inpari 7 and Cibogo varieties, the disease started at temperature 22.3-35.6^{0}C on observation week 2. In Ciherang variety, the blast disease infection rate started at temperatures 22.3-29.4^{0}C on observation week 4 (Table 1). The regression equations used to predict Y variable on each variety of rice are: Y Inpari 7 = -1.3815 + 0.0587x, Y Ciherang = 0.3221-0.0049x, Y Cibogo = -0.8189 + 0.0383x.

The influence of humidity on the rate of blast disease infection

Based on the result of research on the variety of Inpari 7 and Cibogo, the blast disease infection rate started at 69-97% humidity, while on Ciherang variety, the progression of blast disease infection rate started at 85-97% humidity (Table 1). At 69-97% humidity, *P. oryzae* started sporulation. This is supported by Hashioka (1965) which states that sporulation increases in relative humidity above 93% but it rarely occurs in humidity 89-90%, and no sporulation takes place in humidity less than 88% although the spot size is unchanged in high humidity. Differences in the development of blast disease infection rate could be affected by the different resistance of rice varieties to disease and different planting spacing; a denser spacing of rice planting could lead to higher humidity that triggers the blast disease progression. The regression equations used to predict Y variable on each variety are: Y Inpari 7 = -1.3815 + 0.0587x, Y Ciherang = 0.3221-0.0049x and Y Cibogo = -0.0262 + 2.4209x.

The influence of climatic factors on the rate of infection blast

Based on the results of climatic factors observation (temperature, humidity), it was showed that in the varieties of Inpari 7 and Cibogo, the blast disease infection rate was higher than in the variety of Ciherang (Table 4). Differences in the rate of blast disease infection could be affected by different variety resistance to disease, and it can be inferred from the age of the plant at the time of becoming infected. According to Ou (1985), the sensitivity of rice leaves against the infection of *P. oryzae* was associated with the silica content in the epidermis cell wall of the leaf. The lower temperatures and higher humidity and rainfall, the higher the rate of infection blast disease. The regression equation used to predict Y variable on each variety are: Y Inpari 7 = 2.1575 + $0.0193x_1$-$0.02951x_2$, Y Ciherang = -1.9602 + $0.0205x_1$ + $0.0190x_2$, Y Cibogo = 2.0360 + $0.0065x_1$-$0.0203x_2$.

Table 3. The intensity of pathogen *Pyricularia oryzae* attack on the leaves (%) of Inpari 7, Ciherang and Cibogo varieties on observations of 1-12 weeks after planting

Observation on week	Intensity of *Pyricularia oryzae* attack (%)		
	Inpari 7	Ciherang	Cibogo
1	5.0	0.0	3.6
2	16.9	0.0	8.1
3	23.9	0.0	11.3
4	36.1	3.0	19.8
5	42.1	5.1	25.9
6	48.9	6.8	26.9
7	49.1	8.8	28.6
8	49.5	11.9	30.6
9	60.9	13.1	40.1
10	72.0	16.8	46.5
11	76.5	19.8	51.8
12	82.1	23.4	56.6

Note: Data from paddy fields in Tanah Merah North Samarinda, Indonesia

Tabel 4. The rate of blast disease (*Pyricularia oryzae*) infection on Inpari 7, Ciherang and Cibogo

Observation of week	Blast disease infection rate (units/week)		
	Inpari 7	Ciherang	Cibogo
2	1.213	0.000	0.806
3	0.347	0.000	0.325
4	0.414	0.000	0.562
5	0.153	0.535	0.270
6	0.148	0.275	0.038
7	0.005	0.259	0.063
8	0.008	0.305	0.067
9	0.207	0.100	0.270
10	0.168	0.244	0.147
11	0.061	0.165	0.107
12	0.071	0.168	0.090

Note: Data in paddy fields in Tanah Merah North Samarinda

Spot size

Pyricularia oryzae fungus forms spot on rice plants. Morphologically, the typical shape of leaf blast spot is rhombus with two tapered edges (Asyuma 1965; Pasha et al. 2013; Bevitori and Ghini 2015). The fully-developed spot has brown edges and is dark green to bluish gray in color. It reaches a length of 1 to 2.2 cm and a width of 0.3-0.7 cm with brown edges. Spot on the leaves is vulnerable and has no clear edge. The spot is surrounded by yellow color (halo area) especially in a moist environment; in addition, the development of spot is also influenced by the susceptibility of varieties and age of the spots themselves. The spot will not grow and remain as a small dot on the resistant varieties (Figure 1.A). This is due to the development of conidia and *P. oryzae* fungus in the host tissue is inhibited. The spot will grow for up to a few millimeters in round and ellipse shape with brown edges on the susceptible variety (Figure 2.B). This is supported by the opinion of Herawati (1995) that on the sensitive varieties and in humid conditions, spot grows steadily until it reaches the length of 1-1.5 cm and width of 0.3-0.5 cm with unclear brown edge and is surrounded by pale yellow color, while spot on resistant varieties did not develop and remain as a small dot. In a conducive environment, leaf blast can cause death on susceptible varieties from the young plants to the saplings (Figure 2c). In Inpari 7 and Cibogo varieties, the progression of width rate of spot blast disease is faster than in Ciherang variety. Differences in the progression of width rate of spot blast disease could be affected by the resistance of varieties to the disease.

The effect of temperature on the rate of spot width

Spot size is affected by temperature. It is as a result of the growth and development of pathogens. In Inpari 7 and Cibogo varieties, spot blast disease rate is more rapid than in Ciherang variety. It can be said that Ciherang variety is resistant to the attack of pathogen *P. oryzae* (Table 1). The regression equation used to predict Y variable on each variety are: Y Inpari 7 = -0.6869 + 0.0323x, Y Ciherang = 0.3166-0.0026x, Y Cibogo = 0.1232 + 0.0039x.

The effect of humidity on the rate of spot width

Development of a pathogen can be affected by humidity. This leads to the development and the vastness of spot on the leaves which is started by initial symptoms such as small spots which are gradually enlarged into rhombus (Figure 1a). In Inpari 7 and Cibogo varieties, spot blast disease rate is more rapid than in Ciherang variety. It can be said that Ciherang variety is resistant to the attack of pathogen *P. oryzae* (Table 1). The higher the humidity, the greater the rate of spot width of the disease. During the study, the humidity ranged from 69-97%. The regression equations used to predict Y variable on each variety are: Y Inpari 7 = -1.8841-0.0202x, Y Ciherang = -0.7312 + 0.0118x, Y Cibogo = 0.7179-0.0059x.

The influence of climatic factors

Climatic factors (temperature and humidity) and cultivation practices can cause diseases that may be found on leaves with symptoms of a spot in rhombus shape which is commonly called as blast disease. Differences in rates of spot width of leaf blast disease can be influenced by the different resistance of rice varieties to pathogens. Ciherang is a resistant variety to the attack of *P. oryzae* pathogens since the infection occurs when plants are old (Table 5). Plant resistance to blast disease is influenced by plant age (SN et al. 2009). The regression equations used to predict Y variable on each variety are: Y Inpari 7 = 1.2686 + $0.0105x_1$-$0.0163x_2$, Y Ciherang = -2.2434 + $0.0259x_1$ + $0.0213x_2$, Y Cibogo = 1.1698-$0.0077x_1$-$0.0087x_2$.

The number of *Pyricularia oryzae* spores

This disease begins when spores of the fungus infected and produced a spot on the rice plant and end when the fungus sporulated and produced new spores through the air on favorable condition. This may occur one week after the first occurrence and can continue to produce spores for more than 20 days at conducive temperature conditions. High inoculums are very harmful to vulnerable rice plants. The number of *P. oryzae* spores caught by the leaf depends on the wind speed and the position of the leaf or leaf angle. The greater the angle of the leaf the more spores is caught. The number of spores on Inpari 7 variety is more numerous than on Ciherang (Table 6). Differences in the number of spores can be affected by the resistance of rice variety to the disease.

The effect of temperature on the number of spores of *Pyricularia oryzae*

A number of spores can be grown since there is rice variety sensitive to the pathogen and to the effect of temperature. In Inpari 7 and Cibogo varieties, *P. oryzae* spores have already occurred on 7 dap, while in Ciherang variety, *P. oryzae* spores occurred on 28 dap (Table 1). The

Figure 1. Blast disease (*Pyricularia oryzae*) infection. A. Spot leaf disease on Ciherang variety in the form of dots, B. Spot leaf disease forms an unclear edge on Inpari 7 variety, C. The death of the susceptible plant variety (Inpari 7)

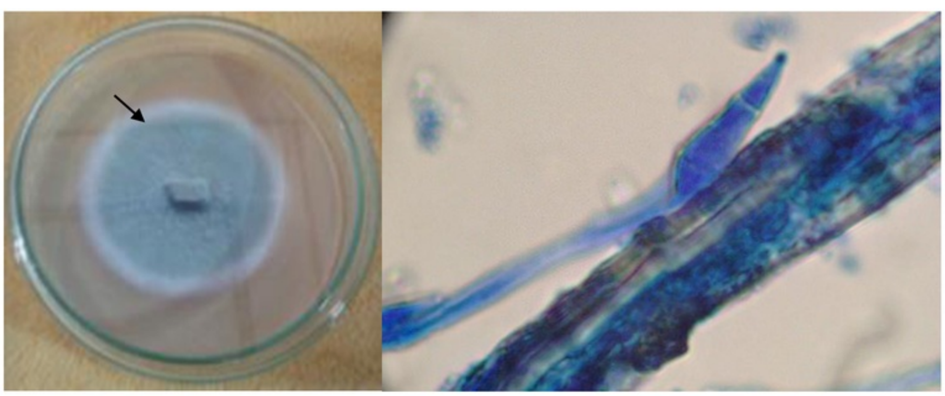

Figure 2. The morphology of hyphae, conidia, and colonies of *Pyricularia oryzae*. A. *P. oryzae* colonies on a petri dish, B. The form of hyphae and conidia of *P. oryzae* in 1000x magnification

Table 5. The size of blast disease (*Pyricularia oryzae*) spot on Inpari 7, Ciherang and Cibogo varieties

Observation on week	Rate of size of blast disease spot (unit/week)		
	Inpari 7	Ciherang	Cibogo
2	0.520	0.000	0.000
3	0.659	0.000	0.859
4	0.227	0.000	0.300
5	0.321	0.827	0.348
6	0.053	0.439	0.091
7	0.181	0.182	0.264
8	0.032	0.337	0.123
9	0.080	0.499	0.068
10	0.148	0.123	0.206
11	0.065	0.068	0.115
12	0.061	0.206	0.179

Note: Data in paddy fields in Tanah Merah North Samarinda, Indonesia

Table 6. Number of *Pyricularia oryzae* spores on Inpari 7, Ciherang and Cibogo varieties

Observation on week	Spore number		
	Inpari 7	Ciherang	Cibogo
1	337	0	0
2	493	0	123
3	593	0	337
4	680	97	493
5	692	168	620
6	694	223	691
7	735	343	593
8	753	381	623
9	832	390	735
10	878	443	753
11	985	585	832
12	998	634	878

Note: Data in paddy field in Tanah Merah North Samarinda

temperature during the study ranged from 22.3-35ºC that supports the occurrence of *P. oryzae* spores. Sporulation can occur at temperatures of 15-30ºC. The optimum temperature for conidial germination and appressorium establishment is 25-28ºC. Appressorium is established after 15 hours of incubation period at a temperature of 20-23ºC (Hashioka 1965). Differences in the number of *P. oryzae* spores can be influenced by the different resistance of rice varieties to blast disease as well as the angle position of rice plants leaves and wind direction. The regression equations used to predict Y variable on each variety are: Y Inpari 7 = 40.0092-0.0165x, Y Ciherang = 31.8618-0.0139x, Y Cibogo = 34.2187-0.011x. If the temperature is equal to zero, the rate of *P. oryzae* spores of Inpari 7 variety is equal to 40.0092, whereas Ciherang variety is equal to 31.8618, and Cibogo variety is equal to 34.2187.

The influence of moisture on the number of spores of *Pyricularia oryzae*

From the three varieties, Ciherang variety has the lowest amount of spores with the development of blast disease infection rate in the humidity of 65-97% (Table 1). Differences in the number of *P. oryzae* spores can be affected by the resistance of rice varieties. The higher the weekly moisture, the greater the number of *P. oryzae* spores. As stated by Ou (1985) that resistance is affected by plant age and the ability to form conidia spots vary depending on the shape and size of the spot. The sensitivity of rice plants against infection of *P. oryzae* has relation with the silica content on the wall of epidermis cell. The older plants have higher silica content than younger plants. The regression equations used to predict Y variable on each variety are: Y Inpari 7 = 52.8226 + 0.0393x, Y Ciherang = 72.5404 + 0.0320x, Y Cibogo = 65.1877 + 0.0289x. If the humidity is equal to zero, then the rate of the number of blast disease spores in Inpari 7 variety is equal to 52.8226, and in Ciherang variety is equal to 72.5404, while in Cibogo variety is equal to 65.1877.

The influence of climatic factors on the number of spores of *Pyricularia oryzae*

Climatic factors (temperature and humidity) and cultivation practices can cause diseases that may be found on leaves with symptoms of a spot in rhombus shape which is commonly called as blast disease. This disease also affects the number of *P. oryzae* spores (Figure 1a). Ciherang is a resistant variety to the attack of *P. oryzae* pathogen, so it has a smaller number of *P. oryzae* spores than Inpari 7 and Cibogo varieties (Table 6). Plant disease occurs because of the variety that is susceptible to pathogens and sensitive to the influence of climatic factors. The regression equations used to predict Y variable on each variety are: Y Inpari 7 = 390.7552-23.5502x_1 + 12.2253x_2, Y Ciherang = 46.2009-29.154x_1 + 12.8588x_2, Y Cibogo = -483.193-24.962x_1 + 21.4267x_2.

The influence of the number of spores on the pathogen attack intensity of *Pyricularia oryzae*

The influence of the spore number on the intensity of the disease on Inpari 7, Ciherang and Cibogo varieties is described here. In Inpari 7 variety, the increasing number of spores is followed by the increasing attack intensity of the disease and it is showed by the increasing number of spores per week. The observation shows that the number of spores and the intensity of the disease attack is highest in Inpari 7 variety and the smallest number of spores and intensity of disease attack is in Ciherang (Table 6), which means that the highest resistance variety is Ciherang and the most vulnerable variety to the attack of *P. oryzae* is Inpari 7. Generally, the more susceptible plant against infection of *P. oryzae*, the higher the attack intensity and the number of spores (Kato 1970). The regression equations used to predict Y variable on each variety are: Y Inpari 7 = -41.8296 + 0.1228x, Y Ciherang = -0.5578 + 0.0353x, Y Cibogo = -3.0277 + 0.0578x.

Biology of *Pyricularia oryzae*

The study of the biology of the pathogen population which is a synthesis of the disciplines of epidemiology and pathogen population genetics (Milgroom 2001; Milgroom and Peever 2003; Titone et al. 2014) is an approach that is based on "*problem-oriented*". Biology of *P. oryzae* begins when spores of fungus infected and produced spots on rice crop and end when the fungus sporulated and produced new spores through the air on favorable condition. This cycle may occur one week after the first occurrence and can continue to produce spores for more than 20 days at conducive temperature conditions. High inoculums are very harmful to vulnerable rice plants. As mentioned by Asyuma (1965) that *P. oryzae* takes 10-24 days to complete one cycle of blast disease and that spotting symptoms is seen four days after inoculation, and 6-7 days later, *P. oryzae* produces conidia for as long as 14 days (Figure 2).

The morphology of hyphae, conidia and colonies of *Pyricularia oryzae*

The isolation of *P. oryzae* used PDA media. The form of *P. oryzae* colonies on a petri dish was in the form of blackish-gray fine threads (Figure 2.A). Conidia appeared on day 16 after the insulation is done. Morphologically, when conidia *P. oryzae* was observed under a microscope, it was oval opaque with two insulations (three chambers) or avocado-like with a small indentation on its end that differentiates it from other conidia. Hyphae of *P. oryzae* were very long that they resembled tangled threads, with no insulation and in the shape of opaque. The size of *P. oryzae*

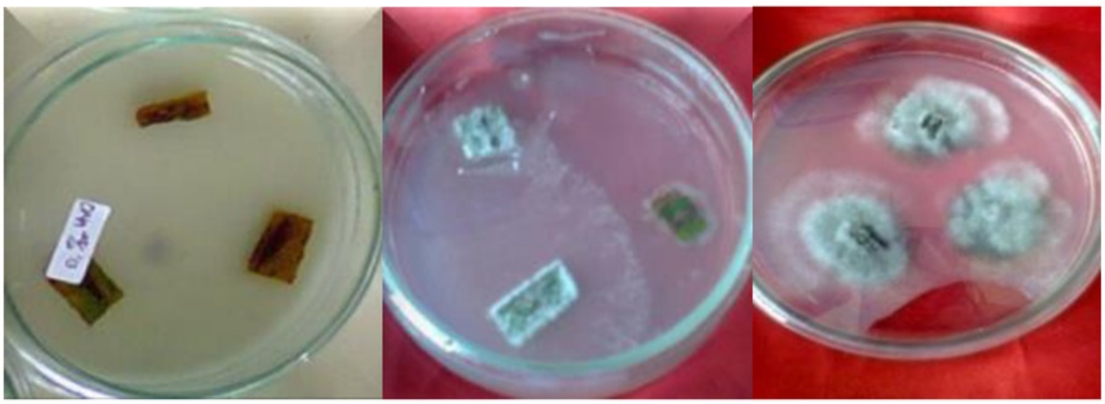

Figure 3. *Pyricularia oryzae* colony growth. A. Observation on day 1, B. Observation on day 4, C. Observation on day 8

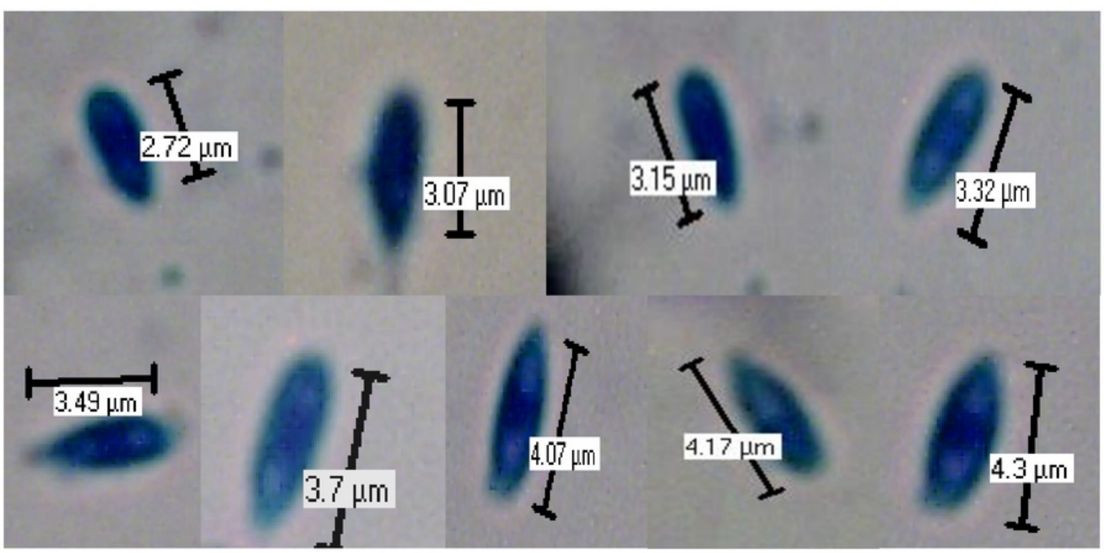

Figure 4. The growth of *Pyricularia oryzae* conidia. A. Observation on 1st day, B. Observation on 2nd day, C. Observation on 3rd day, D. Observation on 4th day, E. Observation on 5th day, F. Observation on 6th day, G. Observation on 7th day, H. Observation on 8th day, I. Observation on 9th day

conidia was very small, and only with the help of a microscope with 400x magnification, the form of conidia *P. oryzae* was quite clearly visible (Figure 2.B).

The growth of *Pyricularia oryzae* colony

The development of *P. oryzae* colony was observed to determine its progress in every observation. In this research, development of *P. oryzae* colonies was observed every four days until conidia were formed. The observations resulted as follows: on the first observation (day 1) (Figure 3a)), the colonies could not be measured since they have not grown yet on the media. On the second observation (day 4) (Figure 3.B), the colonies began to appear, but they were still small spots on the leaves of rice plants with a diameter of 1.2 cm. On the third observation (day 8) (Figure 3.C), the colonies of *P. oryzae* reached the diameter of 2.7 cm. On the fourth observation (day 12), the colonies of *P. oryzae* reached the diameter of 5.3 cm. On fifth observation (day 16), the colonies of *P. oryzae* reached the diameter of 6.3 cm (Figure 2.A). With the indoor temperature ranged 27-33ºC and humidity ranged from 76-80%, the colonies of *P. oryzae* grew until they could not grow anymore on day 16.

The growth of *Pyricularia oryzae* conidia

Based on the research for nine days, the result showed that the development of conidia on the first day was 2.72 μm, the progress of conidia on the second day was 0.35 μm, it was 0.08μm on the third day, it was 0.17 μm on the fourth day, it was 0.17 μm on the fifth day, it was 0.21 μm on the sixth day, it was 0.37 μm on the seventh day, it was 0.10 μm on the eighth day and the development was 0.13 μm on the ninth day (Figure 4).

Milgroom (2001) stated that the difference in conidial development is influenced by temperature and humidity. The temperature in the Laboratory of HPT ranged from 27-33ºC and humidity ranged from 76-80%. According to Ou (1985), temperature affects the development of germinated conidia. Effective temperature for fungal growth ranged from 20-30ºC with a relative humidity of above 90%.

Based on the result of the research, it can be concluded that the most dominant climate factor is temperature. The temperature of 22.3-29.4ºC affects the rate of infection blast disease, the rate of spot width and the development of *P. oryzae* spores. Ciherang is a variety which is resistant to the attack of pathogens *P. oryzae* while Inpari 7 and Cibogo are less resistant varieties.

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