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Plant resistance to leaves and their effects on paddy rice production in Kutai Barat District, East Kalimantan Province, Indonesia

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Abstract. *Akhsan N, Sopialena, Fahrizal. 2019. Plant resistance to leaves and their effects on paddy rice production in Kutai Barat District, East Kalimantan Province, Indonesia. Asian J Agric 3: 41-46.* This study was aimed to evaluate the effect of fertilizer application on the resistance of lowland commercial rice cultivars (*Oryza sativa L.*) against leaf spot diseases in Kutai Barat District, East Kalimantan Timur, Indonesia and to determine the factors influencing the resistance. A field experiment was conducted in rice fields within Long Iram and Linggang Bigung Sub-districts, West Kutai District and the disease identification was performed at the Laboratory of Pests and Diseases, Faculty of Agriculture, Mulawarman University. The field experiment was designed in a split-plot design arranged in a Randomized Block Design (RBD) using four replications. The main plot was fertilizer application (P) consisting of two fertilizer application treatments, i.e., 200 kg.ha^{-1} Urea (p1), and 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK (p2). The sub-plots were varieties (V) consisting of three varieties, i.e., Ciherang (v1), Mekongga (v2) and Inpari 6 (v3). The disease identification was performed by identification of leaf spot disease, isolated from the sample plants using morphological observation under a microscope. The number and density of stomata, intensity of leaf disease infection, and yield of the rice were observed. The humidity was also measured at the time of observation of leaf spot disease intensity. The results showed that different fertilizer treatments did not affect the leaf spot disease intensity, but the varieties affected the disease intensity at 7, 14, 21.35 and 49 days after planting. The number of stomata of *Ciherang*, *Mekongga* and *Inpari 6* varieties was 230,182 and 236 stomata/mm^2, respectively. *Ciherang* variety was more resistant against the leaf spot disease compared to other varieties. Stomatal density does not always affect the intensity of leaf spot disease in lowland rice. There was a correlation between air humidity and the intensity of leaf disease infection. The interaction between fertilization and varieties was significant for the rice yield and the highest yield was obtained by *Ciherang* variety fertilized with 200 kg.ha^{-1} Urea + 200 kg ha^{-1} NPK about 3.58 Mg.ha-1 (grain wet weight).In conclusion, Fertilizer application does not affect the leaf spot disease infection and Ciherang variety is the most resistant plant against leaf spot disease compared to *Mekongga* and *Impari 6* varieties.

Keywords: Fertilizer application, Kutai Barat District, leaf spot disease, plant resistance

INTRODUCTION

Rice (*Oryza sativa* L.) is a cultivated plant that has a very important role in Indonesia for food and other needs. Indonesia is the third-largest rice producer in the world after China and India (FAOSTAT 2014). Rice is a staple food for most people in Indonesia, as well as one of the largest importing countries in the world after Myanmar, Vietnam, and Bangladesh (Oishimaya 2017). With an increasing population in Indonesia, there is an increase in the demand for rice. On the other hand, Indonesian rice production is not still sufficient to fulfill the Indonesian people rice consumption. Indonesian government endorses many efforts to achieve rice self-sufficiency by farming intensification and extensification.

Intensification and extensification of rice cultivation in Kutai Barat District, East Kalimantan Province was carried out in seven sub-districts, i.e., Penyinggahan, Muara Pahu, Mook Manaar Bulan, Barong Tongkok, Linggang Bigung, Long Iram and Bongan. In Long Iram and Linggang Bigung sub-districts, paddy fields have been extended with each planting area of 361.4 ha and 493.9 ha carried out in 2016 (Central Statistics Agency 2016). Furthermore, Long Iram Seberang, and Linggang Amer sub-districts, are two regions that have different altitudes compared to other regions. In these two areas, the planting season is carried out twice a year, since the water supply is abundant to support lowland rice cultivation. Therefore, the rice production in these areas is higher than in other areas.

At present, the pest and disease attacks are increased significantly by the time of the increase of rice area cultivation. Efforts of pest and disease control have been conducted, but those were limited and lacked efficiency. Spotting and exploding diseases are some of the most destructive and are increasing from year to year. In addition, the attention of the Agricultural Agency responsible for pest and disease control in those areas is also still limited (UPTD. Food Crop Agriculture and Horticulture 2017). Rice plants attacked by leaf spot disease will reduce the function of leaves to conduct photosynthesis, consequently, It will reduce the plant yield (Debona et al. 2014).

Disease attacks will occur if there is interaction between susceptible plants, virulent pathogens, and a supportive environment (Semangun 2001). Climate is an environmental factor that supports the interaction of virulent pathogens and susceptible plants. One such climate factor is humidity. The development of pathogens which are generally microorganisms, is strongly influenced by humidity. As a result, the disease will develop well if the humidity level is appropriate (Wiyono 2007). Plant resistance to pathogens is also influenced by nutrients that can strengthen plant tissue. Abdulrachman and Yulianto (2001), stated that application of nitrogen, phosphor, and potassium fertilizer to rice plants could reduce the intensity of brown leaf spot disease from 57.81% to 32.05% and striped spot disease from 8.55% to 2.48%. Suryadi (1995) also reported that the application of potassium fertilizer in rice plants can also reduce the intensity of leaf blight disease by 20-30%, compared to without using the potassium fertilizer application. The plant resistance against leaf spot diseases is also influenced by the genetic properties of the plant itself. Plants that are compatible with pathogens will cause a higher intensity of disease, however, plants that are tolerant to disease can still survive and produce grains (Horsfall and Cowling 1980). Therefore, it is necessary to conduct a study to evaluate the resistance of the plants of three rice varieties to leaf spot disease and rice production and the factors that influence them with different fertilization applications.

MATERIALS DAN METHODS

The study site

The research was conducted at rice farms in Long Iram Seberang, Long Iram, Linggang Amer, and Linggang Bigung Sub-districts of Kutai Barat District, East Kalimantan Province, Indonesia. Identification of leaf spot infected plants was carried out at the Plant Pest and Diseases Laboratory, Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia.

Leaf spot pathogen isolation and identification

The media used for the pathogen isolation and identification was Potato Dextrose Agar (PDA) as an ingredient for the isolation media of pathogenic fungi and methylene blue (Tuite 1969). Isolation was carried out by cutting the leaves between symptomatic and healthy parts, approximately 0.5 cm^2 in leaf area. The leaves were then sterilized with 70% alcohol, washed with distilled water, dried, and placed in PDA media on an aseptic petri dish. Furthermore, the fungus that was growing from the leaves was purified, observed in the microscope, identified, and documented. Finally, the procedures of the Koch postulate were carried out. The identification of the diseases was based on identification key literature for pathogens (Barnett 1960; Booth 1971; Alexopoulos dan Mims 1978; Agrios 1996).

Experimental design

The experiment employed a split-plot design arranged in a Randomized Completely Block Design (RCBD) with 4 replications. The main plot was fertilizer application (P) consisting of (*p1*) 200 kg.ha^{-1} Urea (46% Nitrogen) fertilizer, and (*p2*) 200 kg.ha^{-1} Urea (46% Nitrogen) fertilizer + 200 kg.ha^{-1} NPK (15% Nitrogen, 15% Phosphor, 15% Potassium). The subplot had different cultivated lowland varieties (V), i.e., (*v1*) Ciherang, (*v2*) Mekongga and (*v3*) Inpari 6 varieties. The soil tillage was carried out by hand tractor. The size of an experiment unit plot was 2 x 2 m^2. Transplanting was carried out for 15-20 days of rice seedling in the nursery after sowing. The plant spacing was 25 cm x 25 cm. Twelve plants were determined as samples for each variety, with 6 sample points in each plot. Insecticide and rodenticide were used to control the pest mainly insects and rats. The intensity of leaf spot disease, air humidity, number and density of stomata and rice production were observed.

The intensity of leaf spot infection measurement

The leaf spot infection intensity was observed weekly and 12 observations were carried out during the experiment. The observation was started one week after planting. The infection intensity was calculated using the following formula:

$$I = \frac{\sum (n_i . v_i)}{Z.N} X100\%$$

Note:
I = Infection Intensity (%)
ni= Number of infected leaves
vi= Infection score of infected leaf (Table 1)
N= Total of number of leaves observed
Z = The highest infection score observed (Table 1)

Table 1. Score, and the category of spots infection.

Leaf score	Infection level
1	1 – 5% infection of the total leaf area
3	5 – 11 % infection of the total leaf area
5	11 - 25% infection of the total leaf area
7	25 - 75% infection of the total leaf area
9	75 - 100 % infection of the total leaf area

Source: Directorate of Crop Protection (2007)

After obtaining data on the intensity of rice leaf spot infection, it was followed by calculating the rate of infection of the disease.

Air humidity measurement

The measurement of air humidity was carried out in two research locations starting from the first time of rice planting to the last observation in harvest time using the *HTC-1* digital hygrometer. Furthermore, the relationship between humidity and the rate of disease infection per week was analyzed.

Number and density of stomata observation

The stomata number and density were observed in the Plant Anatomy and Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University, using binocular microscope.

Rice yield

The rice yield was calculated using formula:

$$Y = \frac{10.000\,m^2}{L\,(m^2)} \times \frac{X \quad kg}{1000\,kg}$$

Where:
Y = Rice production ($Mg.ha^{-1}$)
X = Rice yield for each plot (kg)
L = Plot area (m^2)

Data analysis

Data on disease intensity and production were analyzed using Analysis of Variance (ANOVA), and if there were significant differences the post-test of Least Significant Different (LSD) the level of $\alpha = 5\%$ were used to compare the mean values among treatments.

RESULTS AND DISCUSSION

Leaf spot infection intensity

Results of variance analysis on the intensity of leaf spot disease infection in three rice varieties at the age of 7 - 49 days after trans-planting (dat) showed a significant effect, but there was no effect of application of fertilizer treatment. The highest disease infection intensity was observed in the Inpari 6 and the lowest was in Ciherang variety (Figures 1 and 2). Furthermore, at the age of 56-84 dat, both treatments did not give significant effect. There was a tendency for higher intensity of leaf spot disease attack in the treatment of *p2* (200 $kg.ha^{-1}$ Urea (46 % Nitrogen) fertilizer + 200 $kg.ha^{-1}$ NPK compared to *p1* (200 kg ha^{-1} Urea). In addition, the infection intensity of leaf spot disease increased from time to time of observation (Figures 1 and 2).

Results of isolation and identification of leaf spot diseases collected from the study sites found most of diseases were disease-related-fungi. In all varieties, i.e., the leaf spot disease was caused by *Culvularia* sp., *Bipolaris* sp., *Fusarium* sp. and *Pyricularia* sp. (Figure 3). The first *Culvularia oryzae* fungus was reported to cause black grains of rice; this disease is the main disease of upland rice (Busi et al. 2009; Butt et al. 2011; Hsuan et al. 2011), but at the experimental site which is planted in lowland, the diseases were also found and observed. In Pakistan, such kind of disease was also found to cause rice leaf spot disease (Majeed et al. 2016). *Bipolaris* sp., *Fusarium* sp. and *Pyricularia* sp. are very common infecting rice plants (Shabana et al. 2008; Pinciroli et al. 2013; Pagliaccia et al. 2018). According to Ikrarwati and Yukti (2014), the fungi attacking rice especially Ciherang variety in seedling stage are *Alternaria* sp., *Fusarium* sp., *Drechslera* sp., *Synonym Bipolaris* sp., and *Curvularia* sp. The symptom of Fusarium at the leaves shows small dark brown spots and the edges of the spots are light brown and slightly wilted. This fungus is often found in rice plants as well as infecting parts of roots, stems, midribs, leaves, and fruit (Semangun 2001).

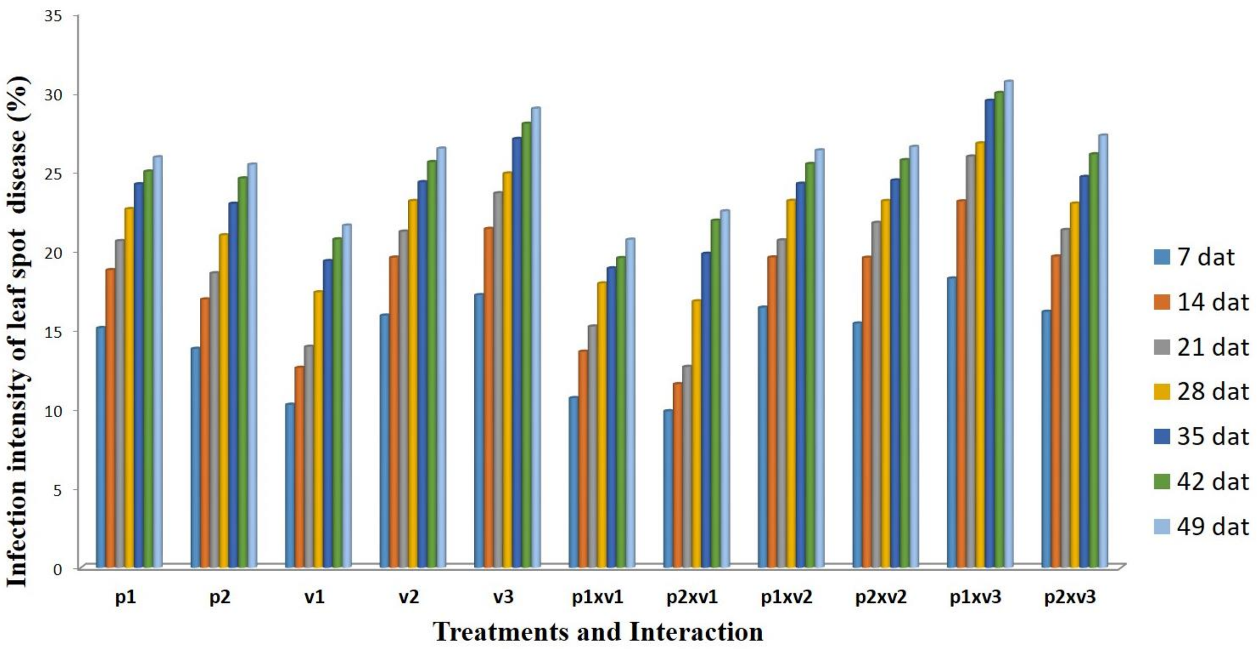

Figure 1. Intensity of leaf spot disease infection on rice plant from 7- 49 dat of observation. *p1*: 200 $kg.ha^{-1}$ Urea, *p2*: 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1*x*v1*, *p2*x*v1*, *p1*x*v2*, *p2*x*v2*, *p1*x*v3*, *p2*x*v2* are interaction between variety and fertilizer application treatment.

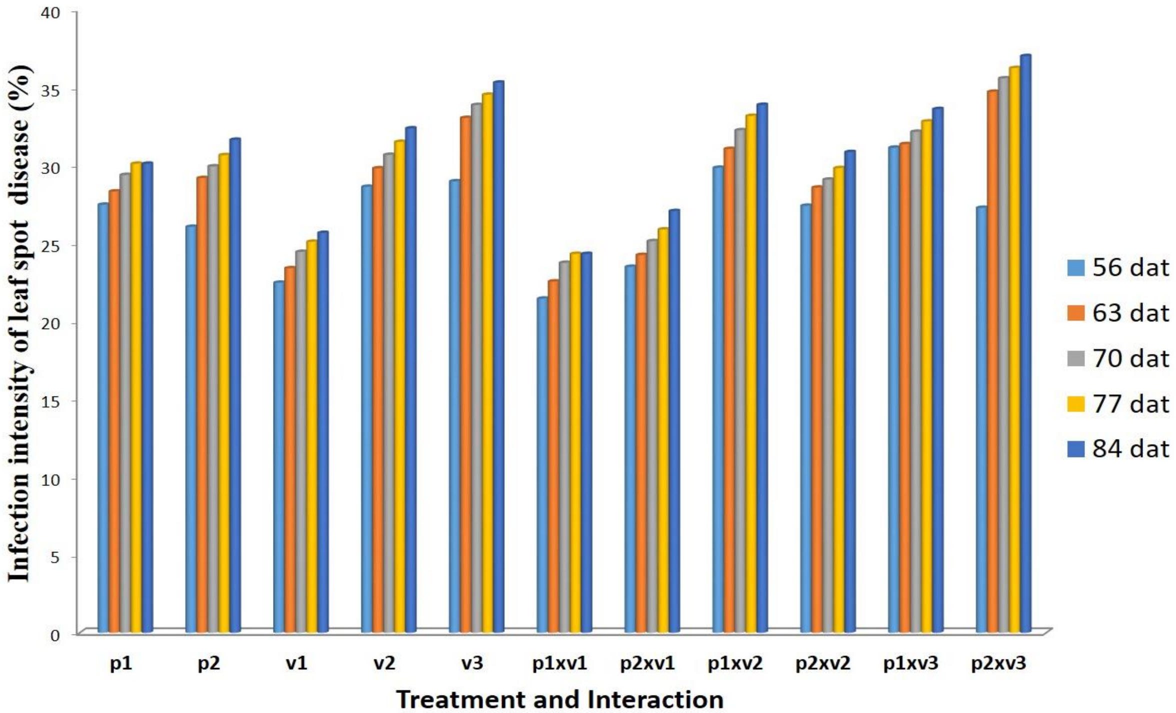

Figure 2. Intensity of leaf spot disease infection on rice plant from 56-84 dat of observation. *p1*: 200 kg.ha^{-1} Urea, *p2*: 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1xv1*, *p2xv1*, *p1xv2*, *p2xv2*, *p1xv3*, *p2xv2* are interaction between variety and fertilizer application treatment.

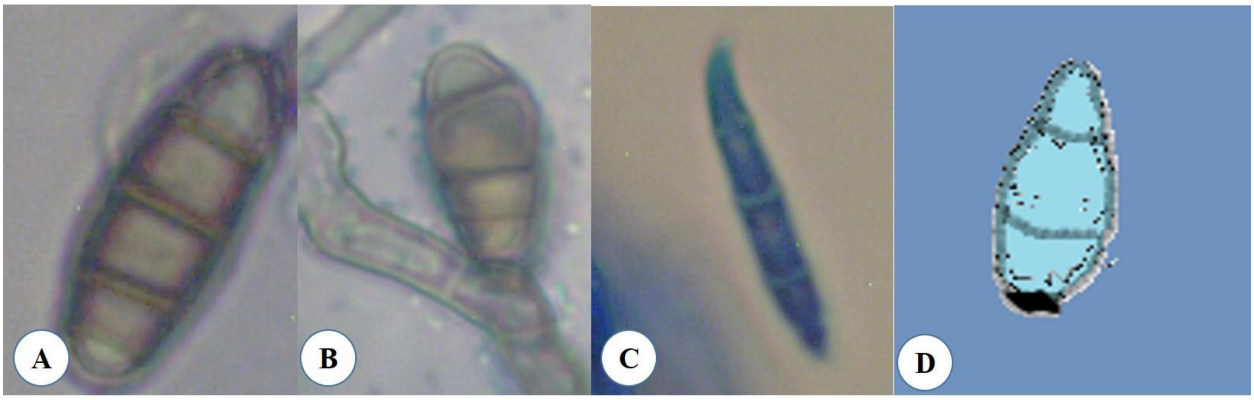

Figure 3. Fungus conidia of *Bipolaris* sp. (A), *Culvularia* sp. (B), *Fusarium* sp. (C), and *Pyricularia* sp. (D).

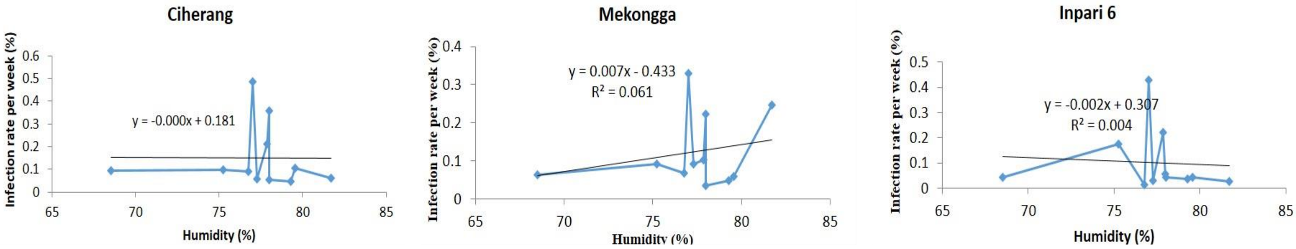

Figure 4. Correlation between air humidity and infection rate of leaf spot disease in Ciherang, Mekongga, Inpari 6 varieties cultivated in Long Iram Sub-district, Kutai Barat District, East Kalimantan Province, Indonesia.

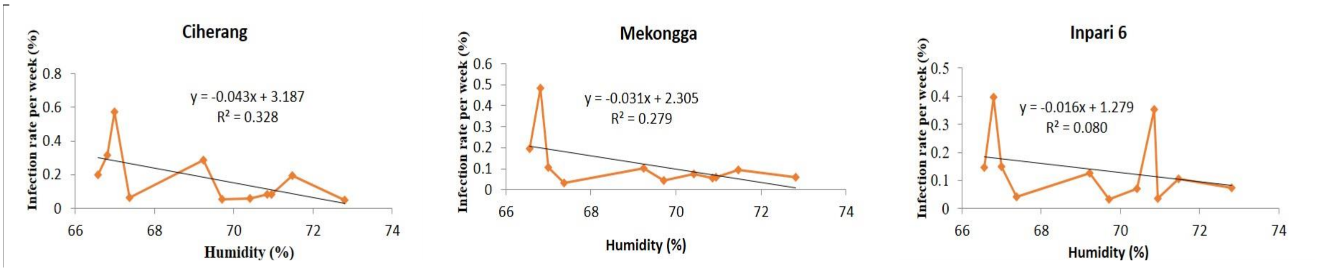

Figure 5. Correlation between air humidity and infection rate of leaf spot disease in Ciherang, Mekongga, Inpari 6 varieties cultivated in Linggang Bigung Sub-district, Kutai Barat District, East Kalimantan Province, Indonesia.

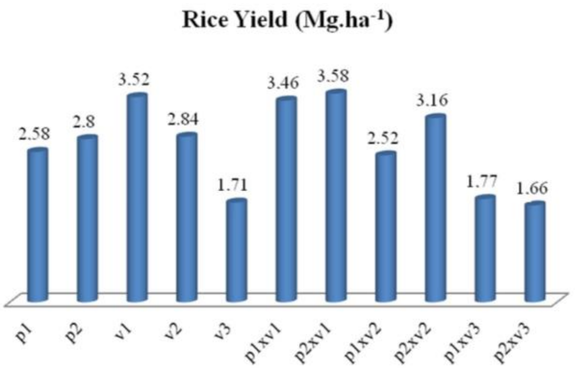

Figure 6. Rice yield due to the variety and fertilizer application associated with leaf spot pathogen attack from 56- 84 dat of observation. *p1*: 200 kg.ha^{-1} Urea, *p2*: 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1xv1*, *p2xv1*, *p1xv2*, *p2xv2*, *p1xv3*, *p2xv2* are interaction between variety and fertilizer application treatment.

Air humidity and the correlation with disease infection intensity

In the concept of triangle disease, climate factors as physical environmental factors that greatly influence the occurrence of disease. Its influence on pathogens can pathogenic life cycle, virulence, transmission, and reproduction (Zeng et al. 2011). Air humidity greatly influences the development, growth, breeding, and reactivity of disease both directly and indirectly. The development of the disease is strongly influenced by the dynamics of climate factors. Relatively high humidity is a potential condition for the onset of disease. The occurrence of pathogenic infections is often determined by the humidity around the plantations, especially for fungal pathogens (Wiyono 2007). The air humidity in these two experimental sites was varied during the study. In Long Iram (altitude 14-16 m asl) air humidity was between 68-83% while in Linggang Bigung (altitude 19-21 m asl) was between 66-73%. The infection rates increased in air humidity from 76 to 78% in Long Iram, and in air humidity from 65 to 67% in Linggang Bigung. The results of the correlation analysis (r) showed that the correlation between air humidity and the rate of infection of rice leaf spot disease in Long Iram was in Ciherang variety r = -0.0092, Mekongga r = 0.2485, and Inpari 6 r = 0.0696 (Figure 4). Furthermore, the correlation between air humidity and the rate of infection of rice leaf spot disease in Linggang Bigung was in Ciherang variety r = -0.5731, Mekongga r = -0.5288, and Inpari 6 r = -0.2843 (Figure 5). These results indicate that the increasing humidity was not always accompanied by an increase in the infection rate of leaf spot disease.

Stomatal number and density

The observations of the stomata density showed that there were 230, 182 and 236 stomata per mm in Ciherang, Mekongga and Inpari6 varieties, respectively. The previous observation concerning the leaf spot disease showed that the highest infection intensity of leaf spot disease was observed in Inpari 6, whereas the lowest intensity was observed in the Ciherang followed by Mekongga varieties. The greater the number of stomata, the greater the possibility of pathogen penetration as showed in Seraiwangi plant tissue (Idris and Nurmansyah 2016). Plant resistance against leaf spot disease is associated with low stomata density. Our results were in line with the study of Pradana et al. (2017), which reported that the higher the density of stomata in the leaves, the more susceptible to penetration or infection of pathogens. Pathogens can penetrate through natural holes in plant, one of which is stomata. The number of stomata can be used as an indicator of plant resistance to a disease, the higher the number of stomata on the leaves, the higher the penetration and pathogen infection to leaf tissue. The fact that the Ciherang variety had high stomatal density, but was more resistant to leaf spot disease is contradictive with the previous study. Therefore, in this case, the stomatal density cannot always be used as an indicator of plant resistance to leaf spot disease. The narrow opening of the stomata acts as a structural defense mechanism (Yudiwanti 2007). Stomata openings are the main route of entry of pathogens into plants and plants have evolved a mechanism to regulate stomata openings as an immune response to bacterial invasion. Closure of the stomata causes fewer pathogens to enter the plant, thus having a negative impact on pathogenesis (Zeng et al. 2011).

Rice yields (Mg.ha^{-1})

The results of the analysis of variance on rice yield due to the interaction effects of varieties and fertilizer application showed a significant effect. The rice production yield is presented in Figure 6. If there is an association between the rice yield with the intensity of leaf spot disease, the low intensity of the disease will produce high yield as observed in Ciherang variety. Conversely, if the high intensity of the disease will produce low rice yield as shown in Inpari 6 variety.

Fertilizer treatment has a significant effect on rice production, but not significantly on the level of disease intensity both in the vegetative and generative phases. Taufik (2011) stated that in generative phase, the resistance response of rice plants varies but there is a tendency that varieties resistant or vulnerable in the vegetative phase also tend to be more resistant or vulnerable at the generative level. The intensity of leaf spot disease was higher the treatment of (*p1*) 200 $kg.ha^{-1}$ Urea compared to 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK fertilizer. This is comparable for rice yield, where the rice yield at the treatment of 200 $kg.ha^{-1}$ Urea fertilizer is lower (2.58 Mg. ha^{-1}) compared to at the treatment 200 $kg.ha^{-1}$ Urea +200 $kg.ha^{-1}$ NPK fertilizer (2.8 $Mg.ha^{-1}$). Walkey (1985) and Dewi et al. (2013) stated that the resistance of plants tolerant to diseases, in general, has good growth and can compensate the level of pathogenic infections to produce high yield. According to Agrios (2005), tolerance or resistance is a trait that can be inherited, these traits allow pathogens to develop and multiply within the host while the host does not have receptor parts to activate toxic substances released by pathogens, so that plants are still able to produce.

In conclusion, from the study, we can see that the resistance of rice against leaf spot diseases is strongly influenced by the varieties. Ciherang variety is the most resistant to the leaf spot disease in the study site at 7- 49 days after planting of observation. More than one pathogenic fungus was identified to cause leaf spot diseases of rice plants cultivated in the study sites. There is a positive correlation between the humidity and the rate of infection of leaf spot disease. Stomata density is not always to be an indicator of plant resistance to diseases. Varied interaction was observed between varieties and fertilization on rice yield. The highest yield was produced by Ciherang variety with fertilizing of 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK (3.58 Mg.ha-1).

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