Virulence diversity of rice blast *Pyricularia oryzae* Cavara

N A Salimah¹, T Kuswinanti², Rianingsih³, A Nasruddin¹ and Baharuddin¹

¹ Student of Postgraduate School, Hasanuddin University of Makassar, Indonesia
² Department of Plant Pest and Diseases, Agriculture Faculty, Hasanuddin University of Makassar, Indonesia
³ Graduated Student of Postgraduate School, Hasanuddin University of Makassar, Indonesia

E-mail: koeswinanti@yahoo.com

Abstract. The purpose of this study was to found the distribution of *Pyricularia oryzae* races in three different regencies in South Sulawesi and to analyze gene linked to the virulence of *P. oryzae* in causing blast disease on rice. Determination of *P. oryzae* races was conducted using 1 set of rice differential varieties consisting of Asahan, Cisokan, IR 64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. The intensity of blast disease was observed seven days after inoculation using the Standard Evaluation System for Rice (IRRI) evaluation standard using a 5-9 scale of the necrotic area to determine the host resistance level. Based on morphological observation, 20 isolates were referred to as *P. oryzae*. Inoculation of *P. oryzae* isolates on 7 differential varieties of rice showed, that 12 races (000, 001, 003, 010, 020, 023, 100, 102, 110, 111, 173, dan 251) existed between *P. oryzae* isolates tested. However, the 020 races were dominant in all districts were samples were collected.

1. Introduction

*Pyricularia oryzae* can cause leaf blast, node blast, neck blast, grain blast and collar blast of rice [1, 2]. Economic losses are incalculable, but some data show a value of more than 70 billion dollars in several countries in Asia [3]. The difficulty in controlling the disease suggested cause by high genetic diversity and highly adaptive cellular and morphological development of *P. oryzae* in infected rice plants [4]. These properties cause the fungus *P. oryzae* races to change virulence in a short time, depending on the host and environmental influences [5]. The development of blast disease in lowland rice is thought to be related to rice cultivation techniques, especially the use of high-dose of N fertilizers, as well as the planting of varieties susceptible to this disease. This phenomenon also occurs in several countries such as Japan, the Philippines, Vietnam, and Korea [6].

Blast disease can be controlled by various ways including cultivation techniques, planting resistant varieties, and the use of fungicides. The use of resistant varieties is the most effective, economic, and easy however, the use of this technology is faced with *P. oryzae* that have high genetic diversity, adapt and easy to form new races that able to break the resistance of newly introduced varieties [7, 8, 9]. The cause of this dynamic population formation is the ability to carry out recombination both sexually and asexually [10]. A number of superior varieties targeted to control blast disease in an environment can only develop for two to three seasons [4].
Research on the distribution of *P. oryzae* races in Indonesia has been carried out. A total of 18 races namely race 001, 021, 040, 041, 051,061, 071, 073, 100, 101, 121, 201, 203, 241, 301, 333, 341, and 343 have been identified in several locations in Indonesia such as Sumatra (Wood Agung, Lampung), Central Kalimantan (Dadahup), Bali (Tabanan) and West Java (Kuningan) from 2007 to 2008 [12]. However, until now there are no data on the distribution of races in South Sulawesi. According to Amir et al., [11], there are four races that exist every planting season, namely race 001, 003, 033, and 173, meaning that this isolate has a widespread. Race isolate 001 has the lowest virulence level of pathogenicity, is widespread and can last long in the field [13]. The determination of new races from blast pathogens in Indonesia is carried out using differential varieties namely Asahan, Cisokan, IR64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali [14]. Therefore, it is necessary to conduct research on the race of *P. oryzae* by monitoring the existence and dominance of *P. oryzae* pathogenic races in South Sulawesi as a basis for recommendations that planting existing varieties resistant to races is very necessary [4].

Genes contained in international standard differential varieties consisting of monogenic strains containing 25 resistance genes in blast disease, namely (Pia, Pib, Pii, Pik, Pik-h, Pik-p, Pik-s, Pish, PIt, Ribbon, ribbon-2, Piz, Piz-5 (= Pi2), Piz-t, Pi 1, Pi 3, Pi5 (t), Pi7, Pi9, Pi11 (t), Pi12 (t), Pi12 (t), Pi19 (t) and Pi20) have been developed and are widely used in several regions [6]. In Indonesia, the monogenic IRBLkh-K3, IRBLz5-CA®, and IRBLz-Fu lines are used which each contain Blas Pik-h, Pi2 (t), and Piz disease resistance genes which have extensive resistance to *P. oryzae* isolates [12]. While the monogenic strains of IRBL-b-B (Pib) and IRBLt-K59 (Pit) showed susceptible reactions to Blas isolates in Indonesia so that their use was ineffective [7]. This variation is closely related to genetic variation which is strongly influenced by a number of pathogenic selection pressures [15, 16].

2. Methods

This research was conducted at the Laboratory of Plant Diseases at the Faculty of Agriculture, Hasanuddin University. The PCR test was carried out at the Research Laboratory, Hasanuddin University Teaching Hospital and sampling would be carried out on paddy rice in three districts representing two climate zones in South Sulawesi namely Bone, Maros, and Gowa.

2.1. Origin and isolation of *Pyricularia oryzae*

Sampling of infected plants were carried out using purpose sampling method on vegetative and generative phases of rice plant. Pathogens were isolated following the standard tissue isolation procedures as described in Tuite [17], whereas identification of the morphology of pathogenic fungi *P. oryzae* was carried out based on Bonman et al., [18].

2.2. Determination of *Pyricularia oryzae* races

Determination of *P. oryzae* race according to the method of Balai Besar Padi, Sukamandi modified by Prabawa et al. [19], using 1 set of differential varieties consisting of 7 varieties: Asahan, Cisokan, IR 64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. The soil preparation following the method from Lestari et al., [20], while preparation of *P. oryzae* inoculum following the method of Listiyowati [21].

The intensity of blast attacks was observed seven days after inoculation using the IRRI evaluation standard [22] as presented in Table 1. Race numbers are given based on the reaction patterns of Indonesian differential rice varieties to the results of inoculation (table 1, [14]). Then calculate the intensity of the disease with the following formula:

\[ S = \frac{\sum (n \times v)}{(N \times v)} \times 100 \% \]

where:

\[ S = \text{Disease severity} \]
n = Number of leaves with a certain score
v = Score of affected leaves
N = Number of leaves observed
V = Highest score scale

Table 1. Pyricularia origin rice number [14]

| Differential Varieties | No code | Races |
|------------------------|---------|-------|
|                        |         | 001   | 003   | 011   | 053   | 153   | 221   |
| Asahan                 | 200     | R     | R     | R     | R     | R     | S     |
| Cisokan                | 100     | R     | R     | R     | R     | S     | R     |
| IR 64                  | 40      | R     | R     | S     | S     | S     | R     |
| Krueng Aceh            | 20      | R     | R     | S     | S     | S     | S     |
| Cisadane               | 10      | R     | R     | S     | S     | S     | R     |
| Cisanggarung           | 2       | R     | S     | R     | S     | S     | R     |
| Kencana Bali           | 1       | S     | S     | S     | S     | S     | S     |

3. Results and Discussion

3.1. Diseases intensity of leaf blast
Observation in the field showed that the highest disease intensity was in the Ciliwung variety with a percentage of 60.66% and 53.36% in Bone and Maros regencies, followed by Cigeulis varieties with a percentage of 30.53% and the lowest was the Ciherang variety 23.6%.

Table 2. The intensity of leaf blast attack at two sampling locations

| Location | Variety  | Intensity Attack (%) | Category* |
|----------|----------|----------------------|-----------|
| Bone     | Ciherang | 29.95                | Heavy     |
|          | Inpari 4 | 28.34                | Heavy     |
|          | Ciliwung | 53.36                | Heavy     |
| Maros    | Cigeulis | 30.53                | Heavy     |
|          | Ciliwung | 60.66                | Heavy     |
|          | Ciherang | 23.6                 | Medium    |

* Category based on Akhasani and Palupi [23]

3.2. Characteristic of blast fungus
Totally twenty isolates were collected from Bone, Maros and Gowa regencies. The colonies were gray to blackish gray with velvety colony texture on PDA medium. Mycelia has a circular shape like a concentric ring that leads to the center. It has a pyriform conidium in which the base is generally round with narrow edges and hyaline. There are two septa with three cells that are widely different (figure 1). Based on these morphological observation, 20 isolates were referred to as Pyricularia oryzae.

Figure 1. Colony appearance of blast fungi. From surface (a), bottom (b) and the shape of conidia (c).
3.3. Race test for *Pyricularia oryzae* isolates

Disease symptoms on rice varieties that are resistant to blast appear in the form of small dark brown spots and the size of the spots do not develop. In susceptible varieties in other hands, it appears rhombus gray spots with both tapered spots. The size of spots on susceptible varieties (S) was develop and coalesce to all parts of the leaves. In moist conditions, the leaves will rot and eventually cause the death of plants.

The race determination of *P. oryzae* based on the results of observations on the intensity of the attack on seven differential varieties found a total of 12 *P. oryzae* fungi composition. Bengo and Lapparija Subdistricts were used as locations for taking samples to represent Bone Regency, Bantimurung, Simbang, and Maros Baru Subdistricts to represent Maros Regency while Gowa District was represented by Patallasang District, Bontonompo District, and Snouts. A total of 5 different racial compositions were found in Bone regency, Maros Regency with 6 different racial compositions and Gowa district found 3 different racial compositions. From the results of race testing (table 3) shows that race 020,023 was found in all locations, while race 001, 100,101,111, 173 and 251 were only found in one sampling location.

| Kode isolat | Ras | Reaksi Varietas padi Diferensial |
|-------------|-----|---------------------------------|
|             |     | AS | CN | IR | KA | CE | CG | KB |
| PoBN1       | 111 | R  | S  | R  | R  | S  | R  | S  |
| PoBN2       | 000 | R  | R  | R  | R  | R  | R  | R  |
| PoBN3       | 001 | R  | R  | R  | R  | R  | R  | S  |
| PoBN4       | 003 | R  | R  | R  | R  | S  | S  | S  |
| PoBN5       | 003 | R  | R  | R  | R  | R  | S  | S  |
| PoBN6       | 020 | R  | R  | R  | S  | R  | R  | R  |
| PoBN7       | 023 | R  | R  | R  | S  | R  | R  | S  |
| PoBN8       | 102 | R  | S  | R  | R  | S  | R  | R  |
| PoMr1       | 173 | R  | S  | S  | S  | S  | S  | S  |
| PoMr2       | 000 | R  | R  | R  | R  | R  | R  | R  |
| PoMr3       | 020 | R  | R  | R  | S  | R  | R  | R  |
| PoMr4       | 101 | R  | S  | R  | R  | R  | R  | R  |
| PoMr5       | 251 | S  | R  | S  | R  | S  | R  | S  |
| PoMr6       | 111 | R  | S  | R  | R  | S  | R  | S  |
| PoMr7       | 000 | R  | R  | R  | R  | R  | R  | R  |
| PoMr8       | 111 | R  | S  | R  | S  | R  | S  | R  |
| PoGw1       | 010 | R  | R  | R  | R  | S  | R  | R  |
| PoGw2       | 020 | R  | R  | R  | S  | R  | R  | R  |
| PoGw3       | 100 | R  | R  | R  | R  | R  | R  | R  |
| PoGw4       | 020 | R  | R  | R  | S  | R  | R  | R  |

AS: Asahan variety  
CN: Cisokan variety  
IR: IR 64 variety  
KA: Krueng Aceh variety  
CE: Cisadane variety  
CG: Cisanggarung variety  
KB: Kencana Bali variety

| AS: Asahan variety | CN: Cisokan variety | IR: IR 64 variety | KA: Krueng Aceh variety | CE: Cisadane variety | CG: Cisanggarung variety | KB: Kencana Bali variety |
|-------------------|---------------------|------------------|------------------------|---------------------|------------------------|------------------------|
| R                 | S                   | R                 | S                      | R                   | R                      | S                      |

4. Conclusions

The isolation of fungi from rice plants in the District of Bone, Maros and Gowa found 20 isolates whose morphological and microscopic characters were identical to *P. oryzae*. Based on the variation of differential rice plant varieties reaction, 12 race pathogens of *P. oryzae* were obtained at three
sampling locations. Race 020 is the most dominant race and is found in three locations.

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