Selection of red rice (Oryza sativa L.) resistant blast disease

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Abstract. Rice crop is one of the major food commodities in Indonesian. However, rice production can decrease due to the blast attack caused by the fungus *Pyricularia oryzae*. Efforts to reduce blast disease can be done by cultivating local red rice. Red rice has high nutritional and antioxidant content and has resistance to blast disease. The aim of research was to determine the level of endurance of 10 lines red rice against blast disease attack and to obtain which rice line resistant to blast disease. The research was conducted from January to March 2020 in the greenhouse of Muara experiment station of Indonesian Center for Rice Research, Bogor, West Java. The research used Factorial Randomized Block Design (FRBD) with two factors. The first factor was 10 lines of red rice, 4 elders and 2 control varieties. Second factor are 4 race blasts (033,073,133 dan 173). The selection method is adjusted to the standard of Interational Rice Blast Nursery (IRBN). The results showed 10 red rice lines were resistant to race blast 033, 9 red rice lines were resistant to race 073, and 7 red rice lines resistant to race (133 and 173). The red rice line; 19i-06-09-23-27, 19i-06-09-23-3, 19i-06-30-17-17 have the highest resistance. The resistance level of these three line was not differed significantly with Situ Patenggang variety (resistant control) and Inpago 8 variety which were the highest resistance to blast disease.

1. Introduction

Rice Crop is one of the major food commodities of Indonesian Society [1]. Average rice weight imported from Vietnam, Thailand, China, Myanmar to Indonesia from 2011-2018 is 1.322.694,438 tons/year [2]. The important type of rice is red rice because there is nutrient content and high antioxidant [3]. Red rice plant in Indonesia lacks the public's attention to be cultivated. This causes the production of red rice is low [4]. Another factor that causes low production of red rice is the blast disease attack.

Blast is a disease caused by fungal *Pyricularia oryzae* [5]. Blast is the most destructive rice disease causing yield losses, which are in staggering dimension [6]. Blast disease can attack all parts and stadia of rice plant growth [7]. Decreased red rice yield due to blast disease can be prevented by using blast-resistant varieties. Rice varieties that are resistant to blast attack can be obtained by various selection methods. Selection to obtain an blast-resistant lines as follows: IRBN (International Rice Blast Nursery) method [8], Tissue culture engineering [9], SSR DNA Marker selection [10], MAS/*marker assisted selection* [11].

The selection method used is the IRBN (*International Rice Blast Nursery*) method is the observation of the symptoms of attack on the leaves of the rice and the scoring in accordance with the *Standard Evaluation System* [12]. The lines of red rice used was the F_6 line of mutant cross M8-GR150-1-9-13 X Inpago 8, M8-GR150-1-9-13 X Banyuasin, Inpago 8 X Balok, x Banyuasin Balok and Balok x Inpago 8 [13]. The line of the red rice is F_6 generation, but crop resistance against the blast attack is not yet

known. The aim of the study is to determine the level of endurance of 10 lines red rice against blast disease attack and to obtain a rice line resistant to blast disease.

2. Materials dan Method

The research was conducted from January to March 2020 in the greenhouse of Muara experiment station of Indonesian Center for Rice Research, Bogor, West Java. The materials used for of the research are F_6 rice seeds and comparator varieties seed (Situ Patenggang, Kencana Bali, Inpago 8, Banyuasin, M8-GR150-1-9-13 and Balok), isolates of *Pyricularia oryzae* fungus (races; 033,133,073 and 173), *oats*, agar, sugar/dextrose, streptomycin, tween 20, aquades, alcohol, water, soil, urea fertilizer, furadan, plastic *wrap*, rubber bracelet, spritus and aluminum foil. The design used Factorial Randomized Block Design with two-factors treatment. The first factor is the varieties/lines of rice consisting of 16 types. The second factor are 4 race blast (033, 073, 133 and 173).

The procedur includes of greenhouse preparation, seed preparation, planting media preparation, planting, sterilization of tools and materials, oat malt agar (OMA) preparation, inoculums preparation, calculation of spore density, inoculation and observation.

Planting of rice seedlings in seeding box with planting medium in the form of soil added urea and furadan. Inoculation is done when the plant is 18-21 days after planting using a spore suspension with density of ± 2 x 10 5/ml. Inoculation is done by spraying spores in rice plants in a direction using auto mizer which is connected with compressor. The observed character are an attack score, intensity of disease (%) and incidence of disease (%). Observations were conducted on 3 DAI, 7 DAI and 14 DAI (days after inoculation) with scoring method (Table 1).

Table 1. Score classification of blast disease attack

Score	Description
0	No lesion observed
1	Small brown specks of pin point size
2	Small roundish to slightly elongated, necrotic gray spots, about 1-2 mm in diameter, with a distinct brown margin.
3	Typical blast (rhombus), 1-2 mm long lesions
4	Typical susceptible blast lesions < 2% of the leaf area
5	Typical susceptible blast lesions 2-10% of the leaf area
6	Typical susceptible blast lesions 11-25% of the leaf area
7	Typical susceptible blast lesions 26-50% of the leaf area
8	Typical susceptible blast lesions 51-75% of the leaf area
9	Typical susceptible blast lesions 76-100% of the leaf area (all plant are dead)

Note: Score 0-2 = Resistant, 3 = Moderately resistant, 4 = Moderately susceptible and 5-9 = Susceptible [12].

The intensity of the blast attack on the rice plant is calculated using the following formula:

$$IS = \frac{\sum n \times v}{\sum N \times V} \times 100\% \quad (1)$$

Note: IS (the intensity of the blast attack), N (the number of clumps affected by leaf blasts), V (the number of attacks of each clump), n (Number of sample plants), v (amount of the highest leaf blast).

Incidence of disease calculated using the following formula:

$$IP = \frac{a}{b} \times 100\% \quad (2)$$

Note: IP; Incidence of disease (%), a (number of sick samples) and b (total sample amount). Incidence of disease is calculated based on the strains/varieties respectively.

The data analysis used F test in 95% significant level and if the data give significant effect it will continue by using Duncan's Multiple Range Test.

3. Result and Discussion

Lines 19i-06-09-23-27, 19i-06-09-23-3, 19i-06-30-17-17, 19i-06-30-17-27, 21b-57-21-21-1, 21b-57-21-21-25, 23a-56-24-22-13, 23a-56-30-25-1, 23a-56-30-25-12, 23a-56-30-25-13 is an F_6 generation red rice lines that has not yet been known resistance level to the blast disease. The lines response against the blast disease are generally varied in various blast races. The rice lines of red rice showing a resistant and stable response can be used to be cultivated and served as a cross-cutting [14]. Selected rice accessions in plant breeding can be developed into blast-resistant cultivars [15]. The result of the resistance test of the red rice line showed that 7 lines that were resistant to 4 blast races namely 19I-06-09-23-27, 19i-06-09-23-3, 19I-06-30-17-17, 19i-06-30-17-27, 21b-57-21-21-25, 23a-56-24-22-13, 23a-56-30-25-12.

Lines 19I-06-09-23-27, 19i-06-09-23-3, 19i-06-30-17-17 selected have a resistance comparable to the resistant control variety (Situ Patenggang) and stable. Score of disease attack and intensity of disease (%) the highest variety is Kencana Bali (susceptible control) and incidence of disease (%) the low is the Inpago 8 variety.

There was a significant difference on the blast resistance among the treatment (Table 2).

Tabel 2. Quantitative characters analysis of red rice lines 16 DAI (day after inoculation) at the age of 5 WAP (weeks after planting)

Parameter	Line		Race		Interaction		CV (%)
	F value	P>f	F value	P>f	F value	P>f	
Intensity of disease (%)	179,97**	<,0001	66,04**	<,0001	2,96**	<,0001	17,59
Incidence of disease (	4,00**	<,0001	4,00*	0,01	4,00**	<,0001	3,56

Note: ** = Significant at the level 1%; *= Significant at the level 5%; tn= Not significant; CV = Coefisien variance; Pr>F= Probability value

The result showed that the rice lines/ varieties have varied response to the attack of the blast disease. The rice lines/ varieties resistance response is indicated by the disease intensity and disease incidence (Tabel 3).

The line of red rice have a varying endurance response to the blast attack based on the attack score mode value. 10 lines of red rice showed resistant to 033 race, 9 lines resistant to 073 race and 7 lines resistant to 133 and 173 races (Table 3). The difference in the response is influenced by the genetic factors of each rice line [16]. Rice genotypes of the same origin have different responses because they are influenced by the genetic makeup differences of each rice genotyping [17].

The durability of varieties can be divided into 2 based genetic vertical resistance (resistant to a single pathogen race) and horizontal resistance (nonspecific resistance to certain races/more than one race and has a stable endurance response) [18]. According to the statement, line of red rice the test results showed that 7 lines of 4 races blast were resistant and 2 lines were resistant to 2 race blast with horizontal resistance while 1 line of race were resistant to have a vertical resistance.

Lines 21B-57-21-21-1, 23A-56-30-25-1 and 23A-56-30-25-13 are not recommended for release as new varieties of blast resistance because the strains show a somewhat resistant and susceptible response to the tested blast races (Table 3). The lines are the result of crosses Inpago 8, Balok and Banyuasin have a resistant, moderately resistant, and susceptible response shown in table 3, so that the results of the elders crossing indicate a somewhat resistant and susceptible response to the blast race. Description of rice varieties that show Banyuasin hold the blast race [19]. Difference response Banyuasin variety suspected endurance genes already broken by the blast race. The varietal endurance response changes to be susceptible due to the genetic diversity and high blast race virulence [20], having one endurance gene [21], the variety resistance is limited by specific place and time [22].

The red rice line; line 19I-06-09-23-27, 19I-06-09-23-3, 19I-06-30-17-17 was not differed significantly with the control group Situ Patenggang variety and Inpago 8 variety on attack scores, disease intensity (%) (Table 3). The resistance response indicated by the lines was suspected due to the presence of a resistant gene from an older variety of Inpago 8 and M8-GR150-1-9-13 shown by Table

3. The directional cross and the superiority of the cross can produce a good seed strain [23]. Cultivars local carry gene resistance to blast diseases [24]. The diversity of local varieties is a factor that causes local rice varieties to be never/rarely severely attacked by blast diseases [25].

Tabel 3. Qualitative character (Attack score) of red rice lines and quantitative characters (Intensity of disease (%) and Incidence of disease (%) of Duncan's Multiple Range Test (DMRT)

Lines/ Varieties	R33		R73		R133		R173		Intensity of disease (%)	Incidence of disease (%)
	S	*R*	*S*	*R*	*S*	*R*	*S*	*R*		
19I-06-09-23-27	1	R	1	R	1	R	1	R	**11.00 f**	100.00 a
19I-06-09-23-3	1	R	1	R	1	R	1	R	**11.00 f**	100.00 a
19I-06-30-17-17	1	R	1	R	1	R	1	R	**11.00 f**	100.00 a
19I-06-30-17-27	1	R	1	R	2	R	2	R	16.88 e	100.00 a
21B-57-21-21-1	1	R	1	R	3	MR	3	MR	24.63 d	100.00 a
21B-57-21-21-25	1	R	1	R	2	R	2	R	16.50 e	100.00 a
23A-56-24-22-13	1	R	1	R	2	R	2	R	17.63 e	100.00 a
23A-56-30-25-1	1	R	3	MR	3	MR	3	MR	24.75 d	100.00 a
23A-56-30-25-12	1	R	1	R	2	R	2	R	18.25 e	100.00 a
23A-56-30-25-13	2	R	2	R	5	S	5	S	36.63 b	100.00 a
Situ Patenggang/ Resistant control	1	R	1	R	1	R	1	R	**11.00 f**	**100.00 a**
Kencana Bali/ Susceptible control	7	S	9	S	9	S	9	S	92.25 a	100.00 a
Inpago 8	1	R	1	R	1	R	1	R	**9.88 f**	**90.00 b**
Banyuasin	3	MR	3	MR	5	S	5	S	46.25 b	100.00 a
Balok	1	R	1	R	3	MR	3	MR	23.13 d	100.00 a
M8-G[illegible]0-1-9-13	1	R	1	R	3	MR	3	MR	24.25	100.0

Note: - Score 0-2 = Resistant (R), 3 = Moderately Resistant (MR), 4 = Moderately Susceptible (MS) and 5-9 = Susceptible (S), Score (*S*) response (*R*) [12].
- The numbers followed by the same letter in the same column show no distinct apparent on the Duncan Multiple Range Test (DMRT) level of the 95%.

Inpago 8 variety different against all lines/varieties of control and elders on the character of the disease incidence (%). Inpago 8 variety is nationally selected variety with resistance to blast races (033, 073, 133 and 173) [19].

Kencana Bali variety (susceptible control) has character score attack and intensity of disease (%) highest compared to other strains/varieties. Kencana Bali variety is used as a susceptible control because it shows the response is very susceptible to all the race of blast disease. The development of the disease in Kencana Bali variety is higher than other varieties [26].

4. Conclusion

Resistance response of the red rice line to the blast disease is 10 red rice lines were resistant to race blast 033, 9 red rice lines were resistant to race 073, and 7 red rice lines resistant to race (133 and 173). The red rice line; line 19i-06-09-23-27, 19i-06-09-23-3, 19i-06-30-17-17 have the highest resistance. The resistance level of these three line was not differed significantly with Situ Patenggang variety (resistant control) and Inpago 8 variety which were well-known the most resistance to blast attack.

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