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West Sumatra Brown Rice resistance to Brown Planthopper and Blast Disease

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Abstract. *Dwipa I, Syarif A, Suliansyah I, Swasti E. 2018. West Sumatra Brown Rice resistance to Brown Planthopper and Blast Disease. Biodiversitas 19: 893-898.* Brown rice is a highly nutritious rice widely consumed as the carbohydrate substitute of common rice. Brown rice resistance to biotic stress is one of indicators of a superior variety. Our study aimed to analyze the response of several brown rice genotypes from West Sumatra to brown planthopper attack and blast disease. This study comprised two experiments, the resistance assay to brown planthopper (*Nilaparvata lugens* (Stal.) and the resistance assay to blast fungi *Pyricularia oryzae*. The resistance assay to brown planthopper was done using randomized block design experiment with three replicates. Eighteen brown rice genotypes (15 brown rice, 2 black rice, and 1 control genotype) were tested in the assay. From 17 brown and black rice tested, 7 genotypes were resistant and 2 were moderately resistant. For blast resistance analysis, fifteen rice genotypes (13 brown rice and 2 black rice) were used. There was only 1 genotype highly resistant and 3 moderately resistant to blast disease among those 15 brown and black rice.

Keywords: Biotic stress, blast disease, brown planthopper, brown rice, West Sumatra

INTRODUCTION

Brown rice is a favorite rice among urban community. Brown rice is nutritious but has lower calory compared to common rice (Varshini et al. 2013). 100 grams of brown rice contain about 7.5 g protein, 0.9 g fat, 77.6 g carbohydrates, 16 mg calcium, 163 mg phosphorus, 0.3 g iron, 0.21 mg vitamin B1, and anthocyanin (Pletch and Hamaker 2018). The awareness of people, especially that of the urban community, on healthy lifestyle results in an increase in the demand for brown rice annually (Babu et al. 2009). Indonesia is no exception, the increase is observed nationwide including in the Province of West Sumatra.

West Sumatra is a region in Indonesia that lies on the Earth's Equator and has a tropical rainforest climate. These conditions make the region rich in exotic genetic diversity resources. One of the important germplasms from West Sumatra is brown rice (Swasti 2004). Swasti et al. (2011) reported 10 local brown and black rice genotypes from West Sumatra. Putra et al. (2010) also reported 9 brown rice from Solok District, West Sumatra. Those reports indicate that West Sumatra possesses plenty of brown rice genotypes potential to be developed into superior rice varieties. One important indicator of a superior crop variety is the resistance to biotic stresses (Yaherwandi et al. 2013). Therefore, the goal of plant breeding program is to develop plant varieties resistant to stresses, pests and plant diseases. Current studies and explorations on local brown rice from West Sumatra barely give us a clear insight on the rice resistant to pests and diseases. Nurhasanah et al. (2018) stated that stress resistance traits of a rice plant are dictated by the genetic makeup of that plant. There is a specific interaction between the host plant and its pathogen in which the resistance gene can render resistance to the pathogen. This resistance to one pathogen is called vertical resistance or when it occurs to more than one pathogen it is called horizontal resistance (Fu et al. 2011; Sekhwal et al. 2015; Nurhasanah et al. 2018). Brown planthopper (*Nilaparvata lugens* (Stal.) is one of the most important pests that has been devastating rice crops, causing a significant yield loss. In West Sumatra, brown planthopper attacks caused a 100% reduction in the rice yield (Taurislina 2015). Not only damage the plant, the brown planthopper is also a vector of rice virus such as rice grassy stunt virus and rice ragged stand virus (Cabauatan et al. 2009). Besides brown planthopper, the blast disease is the primary disease in rice (Babu et al. 2009). Blast disease is caused by *Pyricularia oryzae* Vac. (Hubert et al. 2015). In 2011, blast disease affected 2,208 ha rice field in Indonesia, and increase to 3,649 in 2012 causing a yield loss between 50-90% in particular to susceptible rice varieties (Suriani et al. 2015). In cultivating brown rice, therefore, the loss of yield caused by these two plant pests must be carefully taken into consideration. The first step to generate a brown rice resistant to brown planthopper attack and blast disease is evaluating the tolerance of all available brown rice genotypes in West Sumatra to those pests. Our study aimed to identify local brown rice genotypes from West Sumatra that are resistant to brown planthopper attack and blast disease.

MATERIAL AND METHODS

Propagation of brown planthopper

IR-42 rice seeds (a hopper-susceptible rice variety) were germinated on a seedbed (30 x 20 x 5 cm). 15 days after planting (DAP), rice plants were transferred to plastic pots (diameter 15 cm; height 18 cm) with 4 plants per pot. Urea fertilizer (0.35 g/pot) was applied to 21 DAP-plant. At 30 DAP, rice plants were placed in an insect shield container made of wood covered with milar plastics materials. The base of the insect shield was covered with a sheet of plywood, and the top of the shield was covered with a gauze sheet. There were 5 insect shields (60 x 60 x 60 cm) and each shield contained 6 pots. 10 pairs of adult brown planthopper biotype 3 were placed inside the shield. The IR-42 rice plants were replaced weekly during the experiment (Yaherwandi et al. 2013).

Rice resistance assay to brown planthopper

A rice resistance assay to brown planthopper was done in the screen house and laboratory of Insect Bioecology, Department of Pest and Plant Diseases, Faculty of Agriculture, Andalas University, Padang, Indonesia. The experiment was conducted from October 2012 to January 2013. A Randomized Block Design was used in this experiment. 15 brown rice, 2 black rice genotypes together with 1 control genotypes were analyzed in this study. Each treatment group was prepared in triplicate. The 15 brown rice genotypes were Jorong Mudiak, Padi Ladang, Pido Manggih, Sikarujuik, Gunung pasir, Padi Telur, Surian, Teluk Embun, Kekuningan, Siarang, Pesisir Selatan, Talang Babungo, Sungai Abu, Perbatasan, and Capacino. The two black rice genotypes were Solok dan Beras Hitam Sariak Alang Tigo. Hopper-susceptible IR-42 rice was used as control genotype. All rice plants were grown in the screen house. Rice seeds were germinated on a seedbed (30 x 40 x 5 cm). 14 DAP plants were transferred to pots (diameter 15 cm; height 18 cm), each pot was planted with 1 plant. Urea fertilizer (0.35 g/pot) was applied to 21 DAP-plant. The resistance assay was performed on 30 DAP rice plants. Two parameters observed during the experiment were the rice resistance to brown planthopper and the lifetime of the brown planthopper.

Level of rice resistance to brown planthopper

One brown planthopper was transferred to each pot. Observation began when IR-42 rice started to show a hopper burn symptom. Rice resistant level was determined and classified based on the extent of plant damage as shown in Table 1. Data were analyzed using Duncan's New Multiple Range Test (DNMRT) at a significance level of 5%.

Rice resistance assay to blast disease

The study was conducted in an area endemic of blast disease, Sitiung IV, Dharmasraya District, Province of West Sumatra, Indonesia from March to July 2013. 13 brown rice and 2 black rice genotypes were evaluated in this study. The thirteen brown rice genotypes were Jorong Mudiak, Padi Ladang, Pido Manggih, Sikarujuik, Gunung pasir, Padi Telur, Surian, Teluk Embun, Kekuningan, Siarang, Pesisir Selatan, Talang Babungo, Sungai Abu, Perbatasan, and Capacino. The two black rice genotypes were Solok and Sariak Alang Tigo. A Randomized Block Design was used in this experiment. The resistance assay was conducted in an experimental field of 350 m^2. Each rice genotype was planted systemically in an alternating fashion between rows with a spacing of 20 x 25 cm. The experiment was done in triplicate, with the same sequence of the rice genotype in each replicate.

The observation was done every week on 40-60 DAP plants. Rice plant resistance assay to blast disease was performed following the protocol of IRRI (1996) (Table 2 and Figure 1). Data were analyzed using Duncan's New Multiple Range Test (DNMRT) at a significance level of 5%.

Lifetime of brown planthopper

Gravid female brown planthoppers were transferred to the pot of each rice genotypes and then the lifetime of the hopper's nymphs was observed and recorded. Data were analyzed using Duncan's New Multiple Range Test (DNMRT) at a significance level of 5%.

Table 1. Level of rice resistance to brown planthopper

Score	Symptoms	Range	Resistance level
0	No damage	-	Highly resistant
1	Mild damage, yellow lines appears on the first leaf	≥ 1-3	Resistant
3	The first and second leaves yellow	≥ 3-5	Moderately resistant
5	The leaves yellow, growth inhibited, wilted, and half of the plants are dead	≥ 5-7	Moderately susceptible
7	More than 50% of the plants are dead, and the rest are alive but the growth is stunted	≥ 7-9	Susceptible
9	All plants are dead	≥ 9	Highly susceptible

Source: International Rice Research Institute (IRRI) (1988)

Table 2. Scoring of rice plant resistance to blast disease (based on the shape and color change of the plant (IRRI 1996)

Score	Blast disease symptoms	Resistance
0	No symptoms	-
1	Small brown spots the size of a needle tip; no sporulation	Highly resistant
3	Brown spots 1-2 mm in diameter; necrotic sporulation	Resistant
5	Small ellipse spots (3 mm x 2 mm)	Moderately susceptible
7	Diamond-shaped spots with yellow, brown, or purple margin	Susceptible
9	Overlapping diamond-shaped spots	Highly susceptible

Figure 1. Symptoms and scoring of blast disease effect in rice plant

RESULTS AND DISCUSSION

Rice resistance assay to brown planthopper

Rice resistance to brown planthopper can be assayed by observing the extent of damage suffered by the plant upon the insect attack. Our result showed that there are 8 moderately resistant, 7 resistant, and 2 moderately susceptible rice genotypes (Tabel 3). The difference in the resistance response of the 17 rice genotypes is probably due to the difference in the toxin or antibiotic produced by rice plant (Singh et al. 2017; Qiu et al. 2011). Plants produce substances such as alkaloids or other organic compounds that possess repellent effect to brown planthopper (Sodiq, 2009; Qiu et al. 2011).

The seven genotypes resistant to brown planthopper may have a relatively rigid stem and coarse leaf surface. Srivastava et al. (2014) stated that resistant and moderately resistant varieties have a rather hard stem and coarse leaf surface. The hard and coarse plant structure make the brown planthopper difficult to feed on the plant sap, which eventually leads to nymph death due to starvation. Potassium, Calcium, and Silicone are elements that contribute to the toughness of the plant cell wall structure (lignin and cellulose) (Yaherwandi et al. 2013).

A susceptible genotype corresponds to the lifetime of brown planthoppers that infest the plant. Brown planthoppers with the longest lifetime was observed in IR-42 whereas the shortest was found in Sariak Alang Tigo genotype (Table 4). A resistant plant suppresses the development of pest insects and this property corresponds to plant defense mechanism. Sarao and Bentur (2016) stated that there are three mechanisms of plant resistance: antixenosis (preference and non-preference), antibiosis, and tolerance. We argue that the rice plants that were resistant to the hopper attack have high antibiosis compounds. This, in turn, led to the death of the nymphs in the early phase, abnormal nymph growth, low fecundity, and short lifespan. Previously, Sodiq et al. (2009) have reported insects death in their early developmental phase in resistant genotypes. This effect is presumably due to the presence of active chemical compounds produced by the plant that are toxic to the nymphs. Thus, it is crucial to study these active compounds in brown rice (Ashtiani 2012). Genetic factor affects the brown rice immunity to brown planthopper attack. Du et al. (2009) reported that a rice gene called Bph14 is able to activate salicylic acid, induce callose deposition and boost the trypsin inhibitor production. Altogether these responses suppress the hopper's appetite, inhibit their growth and decrease the hopper's lifespan. Wu et al. (2017) describe the function of another gene, BPH15, contributing for the resistance of rice to brown planthopper. Based on genetic analyses, six miRNAs profile of this gene regulate rice development and defense response to brown planthopper (Wu et al. 2017).

Rice resistance assay to blast disease

In this study, rice plant resistance to blast disease is determined based on its disease index. The disease index as standardized by IRRI (1988) represents the severity of blast disease impact on plant, *i.e.*, the formation of blast spot and its extent. Our result indicates that the responses of the plants on blast disease are varied from susceptible to resistant (Table 5). This difference is influenced by many factors such as temperature, host gene activity, pathogen gene, and other environmental factors (Syakira et al. 2016; Kharisma et al. 2013; Titone et al. 2014). Solok black rice is the only genotype resistant to blast disease of all other genotypes (Table 5). Resistant rice plants usually have a higher silicate content in comparison with susceptible

Table 3. Brown rice resistance to brown planthopper

Genotype	Resistance score	Resistance level
Genotype IR-42 (control)	9,00 a	Susceptible
BrR Jorong Mudiak	6,20 b	Moderately susceptible
BlR Solok	6,20 b	Moderately susceptible
BrR Padi Ladang	5,40 bc	Moderately susceptible
BrR Pido Manggih	5,40 bc	Moderately susceptible
BrR Sikarujuik	4,60 bcd	Moderately susceptible
BrR Gunung Pasir	4,60 bcd	Moderately susceptible
BrR Padi Tlur	4,60 bcd	Moderately susceptible
BrR Surian	4,20 bcd	Moderately susceptible
BrR Teluk Embun	4,20 bcd	Moderately susceptible
BrR Kekuningan	4,20 bcd	Moderately susceptible
BrR Siarang	3,80 cd	Resistant
BrR Pesisir Selatan	3,80 cd	Resistant
BrR Talang Babungo	3,40 cd	Resistant
BlR Sariak Alang Tigo	3,40 cd	Resistant
BrR Sungai Abu	3,00 d	Resistant
BrR Perbatasan	3,00 d	Resistant
BrR Capacino	3,00 d	Resistant

Note: Different letters indicate a significant difference (P> 0.05). *BrR = Brown rice, BlR = Black rice*

Table 4. Lifespan of brown planthoppers in brown rice plants

Genotype	Lifespan (day)	
IR-42 (Genotipe pembanding)	12.40	a
BrR Kekuningan	11.82	a
BrR Pido Manggih	11.80	a
BrR Surian	10.12	ab
BrR Gunung Pasir	9.61	abc
BlR Solok	9.15	abc
BrR Talang Babungo	8.32	abc
BrR Sikarujuik	7.91	abc
BrR Sungai Abu	7.82	abc
BrR Pesisir Selatan	6.88	abc
BrR Padi Telur	6.67	abc
BrR Capacino	6.20	abc
BrR Padi Ladang	5.14	abc
BrR Siarang	5.00	abc
BrR Teluk Embun	4.20	abc
BrR Jorong Mudiak	2.60	bc
BrR Perbatasan	2.40	bc
BlR Sariak Alang Tigo	1.72	c

Note: Different letter indicate a significant difference (P> 0.05). BrR = Brown rice, BlR = Black rice

Table 5. Blast disease index of local brown rice genotypes

Genotype	Disease severity	Resistance level*)
BrR Surian	Severe	Susceptible
BrR Padi Ladang	Severe	Susceptible
BrR Perbatasan	Severe	Susceptible
BrR Kekuningan	Severe	Susceptible
BrR Sikarujuik	Moderately severe	Moderately susceptible
BrR Sungai Abu	Moderately severe	Moderately susceptible
BlR Sariak Alang Tigo	Moderately severe	Moderately susceptible
BrR Gn. Pasir	Moderately severe	Moderately susceptible
BrR Talang Babungo	Medium	Medium
BrR Padi Telur	Medium	Medium
BrR Teluk Embun	Medium	Medium
BrR Jorong Mudiak	Medium	Moderately resistant
BrR Pido Manggih	Medium	Moderately resistant
BrR Siarang	Medium	Moderately resistant
BlR Solok	Mild	Resistant

Note: *): Scoring and classification are based on the Standard Evaluation System for Rice (IRRI 1988); BrR = Brown rice, BlR = Black rice

Table 6. Classification of local brown rice resistance level according to their origin

Origin	Genotype	Resistance level
Solok	BrR Surian	Susceptible
	BrR Padi Ladang	Susceptible
	BrR Talang Babungo	Medium
	BrR Sungai Abu	Moderately susceptible
	BlR Sariak Alang Tigo	Moderately susceptible
	BlR Solok	Resistant
South Solok	BrR Siarang	Moderately susceptible
	BrR Gunung Pasir	Moderately susceptible
	BrR Perbatasan	Susceptible
	BrR Kekuningan	Susceptible
Pasaman	BrR Padi Telur	Medium
	BrR Teluk Embun	Medium
	BrR Jorong Mudiak	Moderately resistant
West Pasaman	BrR Pido Manggih	Moderately resistant
	BrR Sikarujuik	Moderately resistant

plants. Ashtiani et al. (2012) stated that silicate content in rice can protect cell walls from *Pyricularia oryzae* Vac. hyphae. High silicate content physically fortifies rice especially its epidermis cells . Thus, the blast fungus *P. Oryzae* cannot penetrate rice leaf tissue (Buck et al. 2008).

Blast fungi are transmitted via air, attached to leaf surface through water splash, and infect leaf and generate blast spots (Devi and Sharma 2010). Rice resistance to blast disease is determined by the defensive structure of leaves, such as the degree of cuticle wax layer, epidermis-layering cuticle, epidermis cell structure, and the size, shape, and location of stomata and lenticels (Anushree et al. 2016; Soares et al. 2014). Another factor that determines plant resistance to blast disease is the pathogen itself (Verma et al. 2015). We found half of the tested genotypes has the potential to be infected by *P. oryzae*. This result indicates that *P. oryzae* pathogenicity can break the rice defense. Fukuta et al. (2014) stated that rice plant defense is influenced by the genetics of the plant and environmental factors.

Favorable environmental conditions allow rapid growth of *P. oryzae* (Rajput et al. 2017). Bhat et al. (2013) stated that at around 20ºC, rice plants become more susceptible to blast disease. In addition, this situation exacerbates the transmission of the disease. According to the local statistics, the daily temperature at the study location was 21-33ºC (Statistics of Dharmasraya District 2013). Suryadi et al. (2013) stated that the transmission of blast disease will occur much easier in an area with high humidity in comparison to that with dry condition.

Our study found that different rice genotypes from the same origin gave different resistant responses (Table 6). This difference is presumably due to the difference in the genetic makeup of each rice genotype. Genetic factor is the primary determinant of rice resistance to blast disease. Liang et al. (2016) reported *pi66* gene that is able to control blast disease in rice. Fukuta et al. (2014) suggest that many of the wild rice exhibited resistance to blast disease. Different rice genotypes have different morphology (Azizi et al. 2015). This difference is contributed by the difference in the genetics of each rice and environment where it grows. Nurhasanah et al. (2018) reported that in some local rice varieties tested against multiple diseases, there has been a specific interaction between the rice and the pathogen. This interaction is regulated by genes that control the rice resistance. In susceptible genotype, pathogens are flourishing, while in resistant genotype where the plant has a mechanism to recognize and respond to pathogens, the pathogen's growth and propagation are suppressed.

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