The resistance of upland rice doubled haploid lines to blast diseases and brown planthopper

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Abstract. The objective of this study was to determine the resistance of doubled haploid lines of upland rice to leaf blast race 033, 073, 133, 173 and brown planthopper biotype 1, 2 and 3. The experiments were conducted in the Muara greenhouse, Indonesia Center for Rice Research (ICRR), Bogor-West Java Province. All experiments were conducted in the span of October 2017 to April 2018. The materials used in the research were 14 upland rice doubled haploid lines, 2 check varieties namely Inpago 10 and Limboto. Testing the resistance of doubled haploid rice of upland rice to blast disease was coupled with differential varieties i.e Asahan as blast-resistant check variety and Kencana Bali as blast susceptible check variety, while to brown planthopper with differential varieties i.e TN1, Pelita, IR26, IR42, PTB 33 and Rathu Heenati. The experimental design was a randomized complete block design with three replications. Resistance study of doubled haploid lines to leaf blast showed that ST7 and Limboto were resistant to race 033 while ST4, ST7, ST9, ST10, ST12, and Limboto were resistant to race 073. Meanwhile, ST4, ST9, ST10, ST11, ST12, Limboto and Inpago 10 were resistant to race 173. Four doubled haploid lines were moderately resistant to race 133 i.e. ST6, ST12, ST4, and ST14. The highest disease intensity was found in race 133 (52.2%) followed by race 033 (35.9%), race 173 (30.4%) and the lowest in race 073 (28.1%). Based on IRRI method of evaluation, two doubled haploid lines of upland rice i.e. ST1 and ST3 were moderately resistant to the biotypes of brown planthopper.

1. Introduction

Upland rice is among the commodities that can be cultivated in the dry land. The development of upland rice is one of the strategic efforts to increase national rice production. Cultivar suitable for the environment is the key factor in increasing rice yield. Breeding superior upland rice varieties can be done via doubled haploid technology. Doubled haploid (DH) plants have homozygous genetic constitutions, so they can provide benefits in increasing variability and accelerating the breeding of superior varieties [1] [2]. Conventional breeding of superior variety requires 8-10 generations to develop homozygous lines. Doubled haploid technology requires one generation to obtain homozygous population.

Blast disease is an important disease in rice plants that spread in almost all centers of rice production in Indonesia [3], especially in upland rice. The blast can reduce rice yield and threaten global food

reserves [4]. Blast is caused by *Pyricularia grisea* (Cooke), capable of infecting rice plants at various stages of growth from vegetative to generative stages [5]. The blast disease can reduce rice production [6]. Death of rice plants due to blast disease can occur at a vegetative stage. The attack of *P. grisea* on leaf collars decrease in rice production.

Rice varieties showed different responses to leaf blast and panicle neck blast. Testing the resistance of rice varieties to blast disease is important. Test of resistance of rice varieties to blast disease in Pati shows that rice varieties resistant to leaf blast disease are not necessarily resistant to panicle blast. The panicle neck blast disease is more detrimental than the leaf blast [7]. Development of resistant rice varieties to blast that is lasting and has horizontal resistance needs to be done. Monitoring the existence and dominance of pathogenic races as a basis for recommending planting resistant varieties according to existing races is very much needed [8].

The other obstacles to rice production are brown planthopper/BPH (*Nilaparvata lugens* Stal), which is considered as important pest which destroy rice production in Asia [9], including Indonesia. The BPH also acts as a vector of ragged stunt virus and grassy stunt virus [10]. Extensive damage to rice which decreases yield was caused, among others, by population explosion and migration due to the resurgence of BPH, the use of insecticides and the use of susceptible varieties. These could trigger an increase in fecundity, shorter BPH development and growth periods and an increasing proportion of BPH macrophagic forms [11]. In addition, the decline in rice production due to BPH is due to the ability of brown planthoppers to adapt to various environments in a relatively short time so it can easily lead to new biotypes [12]. The objective of this study was to determine the resistance of doubled haploid lines of upland rice to leaf blast race 033, 073, 133, 173 and brown planthopper biotype 1, 2 and 3.

2. Materials and Method

Two experiments have been carried out: 1) resistance evaluation of doubled haploid rice of upland rice to blast disease, 2) resistance evaluation of doubled haploid rice of upland rice to brown planthopper. The materials used in the research were 14 upland rice doubled haploid lines, 2 check varieties i.e. Inpago 10 and Limboto (Table 1). The resistance evaluation to blast disease was coupled with differential varieties i.e Asahan as blast-resistant check variety and Kencana Bali as blast susceptible check variety, while to brown planthopper with differential varieties i.e TN1, Pelita, IR26, IR42, PTB 33 and Rathu Heenati.

2.1. The Resistance of Upland Rice Doubled Haploid Lines to Blast Diseases

The research was carried out in the Muara Greenhouse, Indonesian Center for Rice Research (ICRR), Bogor. Rice seeds were planted directly in the plastic box of 30 cm x15 cm x 5 cm. Fertilizers used were 5 g of urea, 1.3 g SP36 and 1.2 g KCl for every 10 kg of dry soil. *P. grisea* used were races of 033, 073, 133 and 173. Each race of *P. grisea* was cultured on potato dextrose agar (PDA) media on petri dish for 7 days. The pure culture was then transferred to wheat flour agar media for 12 days. On the 10th day after removal, scrubbing fungi colonies was carried out using sterile water containing 0.02 g streptomycin, then stored in an incubator with a 20 watt fluorescent lamp for 48 hours. On the 12th day, a re-scrubbing was carried out using the image brush number 10 and 0.02% sterile water containing Tween 20 to get the spore suspension. Spore density used was 2×10^5 spores.ml^{-1}. Inoculation was carried out by spraying to 18-day-old plants or 4-5 leaf stages. Plants that have been inoculated were incubated for 2 x 24 hours in a humid room, then transferred to the greenhouse. Observations were based on the Standard Evaluation System for Rice [13], namely the tolerance scores of doubled haploid lines to leaf blasts, with the following criteria: resistant (score 0, 1, 2); moderately resistant (score 3); moderately susceptible (score 4, 5), susceptible (score 6, 7), highly susceptible (score 8, 9) and intensity of disease incidence (AI). The formula for calculating incidence intensity is: $AI = y/\delta x$, where: AI = incidence intensity; n = number of affected families; v = score value of each hill; N = number of plants from all hills observed; V = highest score for leaf score.

Table 1. List of upland rice doubled haploid lines

Code	Lines/Varieties*	Code	Lines/Varieties*
ST1	HR-1-12-1-1	ST9	HR-5-7-1-1
ST2	HR-1-32-1-1	ST10	HR-5-9-1-1
ST3	HR-2-22-2-1	ST11	HR-5-9-4-1
ST4	HR-2-27-2-7	ST12	HR-7-15-2-1
ST5	HR-2-34-1-3	ST13	B3-2
ST6	HR-3-6-2-1	ST14	B6-4
ST7	HR-5-11-1-1	ST15	Inpago 10
ST8	HR-5-13-2-2	ST16	Limboto

Note: * HR1: IR83821-99-2-2-2/ I5-10-1-1; HR2: IR85640-114-2-1-3 /I5-10-11-1; HR5: BIO R81/I5-10-1-1; HR7: BIO R81 /O18-B1.

2.2. The Resistance of Upland Rice Doubled Haploid Lines to Brown Planthopper

The research was conducted at Muara Greenhouse, Indonesian Center for Rice Research (ICRR). One rice hill in a 30 cm diameter pot was wrapped in 25 cm diameter mica plastic and 100 cm in height. Twenty-five pairs of brown planthopper (BPH) was inserted for 1-2 night, after which the parent BPH was taken out. It is important to get uniform BPH nymphs. Eggs placed in plants were maintained until instar 2-3. These instars would be used to infest the plants to be tested. Fourteen doubled haploid lines and 2 check varieties were planted in an 80 cm x 60 cm x 15 cm box containing soil. Land in the box made row within 5 cm. Each row was planted with 25 seeds, with three replications. Seeding included check susceptible to biotype 1 (Pelita and TN1), to biotype 2 (TN1 and IR 26), to biotype 3 (TN1 and IR42), check resistant to three biotypes (Rathu Heenati and PTB 33). Then the seedlings in each box were infested with biotype of BPH spread evenly and each stem was infested with 8 nymphs. Scoring the resistance of the lines was conducted in 7-10 days after infestation. Observations were based on the Standard Evaluation System for Rice (SES IRRI 2013): 0 (no damage, highly resistant); 1 (very little damage, resistant); 3 (The first and second leaves of all plants are yellowing, moderately resistant); 5 (yellowing and stunted plants or 10 - 25% plants are withered, moderately susceptible); 7 (more than half of the test plants wither or die and the remaining plants are very dwarf, susceptible); 9 (all test plants wither or die; highly susceptible).

3. Results and Discussion

3.1. The Resistance of Upland Rice Doubled Haploid Lines to Blast Diseases

Fourteen lines of upland rice doubled haploid lines and two national check varieties i.e. Inpago 10 and Limboto, showed varying responses to *P. grisea* race 033, 073, 133 and 173. This is caused by differences in the reaction to the pathogen. Those 4 races of *P. grisea* were found every year [14], Yuliani and Maryana (2014) [15], stated that there were seven races that always appear in each year, namely races 001, 023, 033, 073, 101, 133 and 173. Race 033 is one of the blast races identified and originated from the Subang, Indramayu, Kuningan, Bogor, Sukabumi, and Cianjur [16].

The doubled haploid inoculated using *P. grisea* ras 033 showed that ST7 and Limboto gave a resistant response (score 1), while the seven doubled haploid lines namely ST6, ST8, ST9, ST10, ST11, ST12, ST14 and Inpago 10 gave a moderately resistant response (score 3). Six doubled haploid lines, namely ST1, ST2, ST3, ST4, ST5, and ST13 gave a moderately susceptible response. Asahan as blast-resistant check gave a resistant response to race 033, while Kencana Bali as blast sensitive check gave susceptible response to race 033 (Table 3). Testing of doubled haploid lines to race 073 showed that ST4, ST7, ST9, ST10, ST12, and Limboto gave the resistant response. Seven lines react moderately resistant, namely ST2, ST5, ST6, ST8, ST11, ST13, and ST14, while ST1, ST3, and Inpago 10 variety react susceptible

response (score 5). Asahan as resistant check variety of blast showed resistant response (score 1) while Kencana Bali as blast susceptible check variety reacted highly susceptible (score 9), (Table 2).

Tabel 2. The reaction of upland rice doubled haploid lines to P. grisea race 033, 073, 133 and 173

DH Lines/Varieties	Race 033	Race 073	Race 133	Race 173
ST1	5	5	5	3
ST2	5	3	7	3
ST3	5	5	7	5
ST4	5	1	3	1
ST5	5	3	5	3
ST6	3	3	3	3
ST7	1	1	5	5
ST8	3	3	5	3
ST9	3	1	5	1
ST10	3	1	5	1
ST11	3	3	5	1
ST12	3	1	3	1
ST13	5	3	5	3
ST14	3	3	3	3
Inpago 10	3	5	3	1
Limboto	1	1	3	1
Asahan	1	1	3	1
Kencana Bali	7	9	7	7

Note : 1= Resistant 3= moderately resistant, 5= moderately susceptible, 7=susceptible, 9=highly susceptible

ST4, ST6, ST12, ST14, and Asahan reacted moderately resistant to race 133. None of the doubled haploid lines was resistant to race 133 (Table 2). The doubled haploid lines tested were thought to have no strong resistance against blast race 133. This might be due to the lack of nutrients (silicates) that affect resistance to blasts. Silicate is one of the nutrients that affect plant resistance to disease (including blast) [17]. Race 133 is a race of blast with high virulence traits. Race population monitoring to date has used conventional methods, using 1 set of differential varieties. Seven Indonesian differential varieties are Asahan, Cisokan, IR64, Krueng Aceh, Cisadane, Cisanggarung, and Kencana Bali. More than 30 *P. grisea* races and 133 races were identified as high virulence blasts.

Variable intensity describes the magnitude of disease incidence in a line. Determination of the resistance characteristics of each line was carried out at 9 days after inoculation (DAI) because the highest incidence occurred at 9 DAI. The results of observation of incidence intensity showed that some lines inoculated with race 073 showed the lowest percentage value of attack intensity compared to lines inoculated with race 033, 173 and 133. The average incidence intensity of *P. grisea* race 033, 073, 133 and 173 was found in Table 3. The highest intensity was shown by lines inoculated with race 133. There were four lines with mild intensity in blast race 173: ST4 (9.3%), ST9 (9.6%), ST11 (8.5%) and ST12 (10.0%). Seven lines showed moderate intensity: ST1 (18.9%), ST2 (17.0%), ST5 (22.2%), ST6 (16.3%), ST8 (20.4%), ST13 (24.4%) and ST14 (14.1%) inoculated by race 173. Inpago 10 (10.0%) and Limboto (8.5%) were also resistant to race blast 173, as Asahan as a blast-resistant check showed mild nature (resistant) while Kencana Bali as a sensitive blast check showed heavy intensity (vulnerable).

The high or low intensity of disease is an interaction between plants, pathogens, and hosts [18]. Plants susceptible to disease and the presence of pathogenic agents that have very virulent properties

and are supported by hosts that match the pathogens will cause an increase in disease incidence in plants. Host incompatibility might be a factor inhibiting the development of disease attacks. The difference in incidence intensity on the inoculated lines depends on the agro-ecological conditions and differences in the genetic characteristics of the lines evaluated [19].

Table 3. Incidence intensity of blast disease race of 033, 073, 133 and race 173 in DH lines

DH Lines	Incidence Intensity (%)							
	Race 033	React	Race 073	React	Race 133	React	Race 173	React
ST1	35.9	S	26.7	S	30.4	S	18.9	MR
ST2	30.0	S	18.9	MR	41.9	S	17.0	MR
ST3	28.9	S	27.4	S	29.7	S	29.6	S
ST4	29.6	S	10.4	R	23.7	MR	9.3	R
ST5	32.6	S	16.3	MR	39.7	S	22.2	MR
ST6	20.0	MR	14.1	MR	14.2	MR	16.3	MR
ST7	17.4	MR	17.8	MR	48.9	S	30.4	S
ST8	21.1	MR	20.0	MR	52.2	S	20.4	MR
ST9	11.5	MR	10.7	R	24.3	MR	9.6	R
ST10	12.2	MR	11.9	MR	28.8	S	10.7	R
ST11	15.6	MR	24.8	MR	31.4	S	8.5	R
ST12	12.2	MR	10.4	R	15.1	MR	10.0	R
ST13	27.0	S	24.8	MR	40.5	S	24.4	MR
ST14	17.0	MR	25.6	MR	23.1	MR	14.1	MR
Inpago 10	11.9	MR	28.1	S	23.2	MR	10.0	R
Limboto	8.1	R	10.4	R	25.9	MR	8.5	R
Asahan	10.7	R	11.5	R	25.2	MR	7.4	R
Kencana Bali	60.7	HS	65.2	HS	81.5	HS	68.1	HS

R= Resistant; MR = Moderately Resistant; S = Susceptible; HS= Highly Susceptible

3.2. Resistance of Upland Rice Doubled Haploid Lines to Brown Planthopper

Information on the resistance of rice lines or varieties to pests and diseases is needed. Sitaresmi *et al.* (2013) [20] stated that information on the resistance of varieties to pests and diseases of rice plants is beneficial for breeders to obtain donor genes in the development of resistant varieties. The resistance of rice varieties to brown planthopper has been known to be controlled by resistance genes annotated as Bph for dominant genes and bph for recessive genes. Different resistant varieties can have different resistance genes [12]. ST1, ST2, ST3, ST4, ST13, and ST14 were moderately resistant (Table 4). TN1 and Pelita as BPH sensitive checks showed highly susceptible. Rathu Heenati as a resistant check of BPH showed moderately resistant, while PTB33 showed highly resistant.

ST1, ST3, and ST13 were the lines that reacted moderately resistant to BPH biotype 2. The estimated median value of the three doubled haploid lines to biotype 2 was 3.3. Six doubled haploid lines, namely ST2, ST4, ST5, ST10, ST12, and ST14, showed moderately susceptible reactions. Five doubled haploid lines namely ST6, ST7, ST8, ST9, and ST11 reacted susceptible (Table 4). PTB33 as resistant check for BPH showed a highly resistant response to biotype 2. Rathu Heenati showed moderately resistant to biotype 2. TN1 and IR 26 as susceptible varieties to BPH biotype 2 showed highly susceptible responses.

Table 4. The reaction of Upland Rice Doubled Haploid Lines to BPH Biotype 1, Biotype 2 and Biotype 3

DH Lines	Modus Biotype 1	Criteria Biotype 1	Modus Biotype 2	Criteria Biotype 2	Modus Biotype 3	Criteria Biotype 3
ST1	3	MR	3	MR	3	MR
ST2	3	MR	5	MS	5	MS
ST3	3	MR	3	MR	3	MR
ST4	3	MR	5	MS	5	MS
ST5	5	MS	5	MS	5	MS
ST6	7	S	7	S	7	S
ST7	7	S	7	S	7	S
ST8	7	S	7	S	9	HS
ST9	7	S	7	S	7	S
ST10	5	MS	5	MS	7	S
ST11	5	MS	7	S	7	S
ST12	5	MS	5	MS	9	HS
ST13	3	MR	3	MR	5	MS
ST14	3	MR	5	MS	5	MS
Inpago 10	5	MS	5	MS	7	S
Limboto	5	MS	5	MS	9	HS
TN1	9	HS	9	HS	9	HS
Pelita	9	HS	-	-	-	-
IR 26	-	-	9	HS	-	-
IR 42	-	-	-	-	9	HS
PTB33	1	HR	1	HR	1	HR
Rathu Heenati	3	MR	3	MR	3	MR

Note : MS= Moderately Susceptible, ,MR=Moderately Resistant, HR= Highly resistant, S=Susceptible, HS=Highly susceptible

Evaluation of upland rice doubled haploid lines to BPH biotype 3 showed that 2 doubled haploid lines namely ST1 and ST3 reacted moderately resistant. ST1 and ST3 were assumed to have the same resistance gene. A total of 5 doubled haploid lines namely ST2, ST4, ST5, ST13, and ST14 reacted rather vulnerable, 5 doubled haploid lines reacted susceptible and 2 lines reacted highly susceptible (Table 4). PTB33 and Rathu Heenati showed resistant and moderately resistant responses to BPH biotype 3. TN1 and IR42 as BPH sensitive checks of biotype 3 showed highly susceptible responses. IR42 has no selection power when dealing with IR42 colonies (as host) of brown planthopper [21].

ST1 and ST3 gave moderate resistant reaction to three biotypes of BPH. Naturally, plants have resistance to herbivores [22]. This resistance may be used by breeders to develop BPH resistant rice varieties. In addition to the nature of resilience, preference for alighting and eating activity of brown plant hopper is also important. Brown planhopper has a tendency to alight on susceptible varieties such as TN1 which do not have resistance genes and on IR42 host varieties [22]. At the beginning of release into confinement, BPH perched on rice plants randomly, then gradually moved to the preferred varieties, the more susceptible varieties.

4. Conclusion

From blast resistance evaluation, there were 5 DH lines reacted resistant to two blast races, i.e. ST4, ST9, ST10, and ST12, resistant to race 073 and 173, while ST7 resistant to race 033 and 073. There were DH lines, i.e ST6, and ST14 that reacted moderately resistant to 4 races, and ST8 to 3 races of *P. grisea*. From BPH resistance evaluation, DH lines that reacted moderately resistant to all biotype were ST1 and ST3. DH lines of ST13 reacted moderately resistant to BPH biotype 1 and 2, while ST2, ST4, and ST14 reacted moderately resistant to BPH biotype 1.

References

[1] Dewi IS, and Purwoko BS 2012 *J. Agro Biogen* **8** (2): 78-88
[2] Purwoko BS 2017 Pengembangan teknik kultur antera dan aplikasinya pada percepatan pemuliaan tanaman padi di Indonesia. *Dalam*: Sudarsono, Purwoko BS, (eds.). *Landasan Teoritis dan Penerapan Genetika serta Rekayasa Lingkungan untuk Peningkatan Produksi Tanaman.* IPB Press, hlm. 135-182
[3] Sudir A, Nasution Santoso dan Nuryanto 2013 *Iptek Tan Pangan* **9** (2):85-96
[4] Wicaksono D, Wibowo A, Widiastuti A 2017 *J. HPT Tropika* **17** (1): 62-69
[5] Hajano J, Pathan MA, Rajput QA, Lodhi MA 2011 *IJAVMS* **5**(1):53-63
[6] Soares LCDS, Raphael JPA, Bortolotto RP, Nora DD, Gruhn EM 2014 *Brazilian J of Applied Tech. for Agric Sci.* **7**(2):109-119.
[7] Yulianto 2017 *Iptek Tanaman Pangan* **12** (1): 25-34.
[8] Koizumi S 2009 Monitoring of blas races to ensure durability of blast resistance in Japanese rice cultivars p.1-9 *In* Yoshimichi Fukuta, Casiana M. Vera Crus and Kabayashi N (Ed.) Development and Characterization of Blast Resistance Using Differential Varieties in Ric. *JIRCAS Working report* No. **63**. Tsukuba, Japan
[9] Bottrell DG, Schoenly KG 2012 *J. Asia. Pac. Entomol* **15**: 122-140
[10] Chen Y 2009 Variation in planthopper rice interactions: possible interactions among three species? *In* Heong KL dan Hardy B (eds.). *Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia*. Philipines: International Rice Research Institute.
[11] Nanthakumar M, Lakhsmi VJ, Bhusan VS, Balachandran S, and Mohan M 2012 Decrease of rice plant resistance and induction of hormosis and carboxylesterase titre in brown planthopper by xenobiotics *Pesticide Biochem and Physiology* **102**:146-152
[12] Baehaki SE dan Munawar 2012 *J. Entomologi Indonesia* **10** (1): 7-17
[13] IRRI (2013) *Standard evaluation system for rice* IRRI, Los Banos
[14] Santoso, Nasution A 2009 Pengendalian penyakit blas dan penyakit cendawan lainnya *in* Daradjat AA, Setyono A, Makarim AK , Hasanuddin A. (Ed.) *Buku Padi Inovasi Teknologi Produksi* Balai Besar Penelitian Tanaman Padi. Badan Penelitian dan Pengembangan Pertanian Hal. 531563
[15] Yuliani D, Maryana YE 2014 Integrasi teknologi pengendalian penyakit blas pada tanaman padi di lahan sub-optimal *Prosiding Seminar Nasional Lahan Sub Optimal* 835–845
[16] Nasution A, Santoso, Sudir 2013 Pemetaan ras blas (*Pyricularia grisea*) yang menyerang padi sawah di daerah Jawa Barat. Buku 2. hlm. 1093-1104. *Di dalam*: Abdulrachman S, Pratiwi GR, Ruskandar A, Nuryanto B, Usyati N, Widyantoro, Guswara A, Samita P, Mejaya MJ, (Ed.). *Prosiding Seminar Nasional Inovasi Teknologi Adaptip Perubahan Iklim Global Mendukung Surplus 10 Juta ton Beras Tahun 2014* Badan Penelitian dan Pengembangan Pertanian Kementerian pertanian
[17] Romero AF, Munévar, and Cayon G 2011 *Agronomia Colombiana* **29** (3):473-480
[18] Nurhayati 2011 *Epidemiologi penyakit tumbuhan* Buku Edisi 1 Universitas Sriwijaya 102 hlm
[19] Asfaha MG, Selvaraj T, Woldead G 2015 *Int. J of Life Sc* **3**(4):271-286
[20] Sitaresmi T, Wening RH, Rakhmi AT, Yunani N dan Susanto U 2013 *Iptek Tan. Pangan* **8** (1):22-30

[21] Baehaki 1987 Koefisien seleksi berbagai varietas terhadap wereng batang coklat populasi IR 42 (Deli Serdang) *Seminar Balittan Sukamandi* 7p
[22] Rahmini P, Hidayat, Ratna ES, Winasa IW, Manuwoto S 2012 *J. Penelitian Pertanian Tanaman Pangan* **31** (2) : 117-12