Field screening for blast resistance gene donors, among rice mutant lines of Mira-1

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Abstract. Rice blast disease, caused by *Pyricularia grisea* was one of the most destructive fungal diseases in rice worldwide. Therefore, resistance breeding requires continuous efforts to enrich the reservoir of resistance rice lines to effectively tackle the disease. Mutation induction of the Mira-1 rice variety by gamma rays has been conducted, and M6 generation of rice mutant lines was used for this experiment. The rice mutant lines were selected from a total 112 number of rice mutant lines and it had been planted at Cikembar, Sukabumi Village. Cikembar is a hot spot area of blast disease which was used by researchers for the identification of rice lines resistant to blast, and plants were naturally infected. Disease progress was recorded as leaf and neck blast from 0 to 9 (highly resistant to highly susceptible). The results showed that disease severity was significantly different in the rice mutant lines studied and it was consistent and not affected by the plant arrangement in the field. Five rice mutant lines showed high resistance to blast disease, and it found 25 numbers of rice mutant lines scored 1 or resistant to blast disease, meanwhile, their wild type of Mira-1 rice variety showed susceptibility to leaf blast and neck blast diseases respectively. The agronomic traits of rice mutant lines were better than their wild type of Mira-1 rice variety. It was found that Mi-37, Mi-77, the Mi-82 rice mutant lines were the best lines for the gene donors of rice-resistant mutant line.

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world, providing a staple food for nearly half of the global population. This commodity is a pillar of Food security and sustainability [1,2]. The demand for rice is increasing due to the steadily increasing population in Asia, Africa, and Latin America [3-5].

In Indonesia, according to the Indonesia Statistical Centre, rice consumption for each person is 130kg/per person/per year and it is needed 39.6 million tons/per year of rice for 267 million of the Indonesian population [5]. It is needed to increase the yield and also minimize

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the yield loss caused by challenges of climate change, environmental stress, pest and disease infestation [6].

Rice blast disease, caused by *Pyricularia grisea*, *Pyricularia oryzae*, *Magnaporthe grisea* are the most destructive fungal disease in rice worldwide [7-11]. They can attack any part and any stage of rice growing and it will significantly effect on the decrease of rice production, sometimes reaching 30 - 50 % of rice yield [12]. The infected plants turn pale yellow and exhibit chlorosis and abnormal elongation, poor grain ripening such as empty panicles. In extreme cases, infected plants fall and die [13]. These losses increase the global rice price and reduce consumer welfare and food security. The development of rice tolerant to blast disease is very urgent and very effective in protecting the plants from the diseases.

Mutation induction has proven to be a useful method for introducing new traits that may result in crop improvement and can be used as a complementary tool in plant breeding [14, 15]. Mutation induction by gamma rays has contributed to improving crops, this technique has played an influential role in increasing the world food security and it was successfully used for generating novel characteristics of direct importance in rice production and of potential quality and nutrition [16-19]. According to the database of the Atomic Energy Agency (IAEA), 3,222 officially released mutant varieties worldwide in than 200 crop species [20]. Gamma rays produce Reactive Oxygen Species (ROS), which interact with DNA and cause oxidative damage such as base modifications and single/double-strand breaks. The modifications base can be deleted, inversion and substitution and plants can be mutated to dwarf, drought tolerant, pests, diseases resistant plants [21]. Radiation mutagenesis causes various chromosomal DNA alterations in plants, such as deletions, point mutations, and inversion [19]. Gamma rays are a physical mutagen that appears to be valuable tools for functional genomic research in rice, they have been used in crop breeding programs and classical genetic plant studies, and some of the gene products were probably involved in defense against blast infection.

The use of resistant varieties is the most efficient way to control this disease. Resistant varieties will be able to maintain yields and reduce pesticide use. To create resistant varieties, information about resistance line/variety as a candidate of parental is needed. Therefore it is necessary to test the resistance level of rice mutant lines. The research aimed was to determine the resistance level of rice mutant lines of Mira-1 rice variety from mutation induction of gamma rays and to obtain a new rice variety with high productivity and resistance to blast disease.

2 Material and methods

2.1 Plant material

112 number of M6 generation of Mira-1 rice mutant lines obtained from mutation induction by gamma rays with the dose of 100 Gy used in this research.

2.2 Site selection

The field experiment was conducted at the blast hot spot area in Cikembar, Sukabumi during rain-fed conditions. The area of the experiment is the natural blast spot area and the pathogen will come out during the rainy season.

2.3 Experimental setup

Natural spread of the pathogen in the test lines was allowed from border lines/inoculum rows planted around the nursery. Before planting of test entries, the susceptible rice variety IR64 was planted for one month, and in rainy and humid conditions the conidia of the pathogen were available in the air. These inoculum rows received conidia from the air and started to sporulate. Several 112 rice mutant lines, the Cisokan rice variety as a susceptible control, and Limboto rice variety used as a resistant to blast disease, and Mira-1 as their wild type used for the experiment. About 30-gram seeds of each number of rice mutant lines together with rice control were sown in dry areas. To obtain the effect of blast disease on all the plants, rows of spacing for rice planting was 10 cm x 10 cm. The experiment was conducted 3 times for replication. Chemical fertilizer by 100: 50 : 0 kg NPK/ha was applied as basal dose. The spreader rows were watered in the evening before sunset if there were no rainy days. The experiment was conducted with 3 replications.

2.4 Disease assessment

Disease scoring was done using the Standard Evaluation System (0-9 scale) for rice (IRRI, 1988). Blast symptoms were evaluated on leaves before the booting stage using a 1–9 scoring system as follows : 0 (Highly resistant = HR); 1 (Resistant = R); 3 (Moderately Resistant = MR); 5 (Moderately Susceptible = MS); 7 (Susceptible = S), 9 (Highly Susceptible = HS). The same scoring was also conducted for neck blast disease observation**.**

3 Results and discussion

Pathogenic fungus *P. grisea* can attack rice plants in various stages of growth from seed to panicle growth phase (generative). In vegetative plants usually pathogens infect the leaves, called leaf blasts. In the generative stage, besides infecting the leaves, it also infects the panicle neck, called a neck blast. Table 1 displays of severity of rice mutant lines toward leaf blast disease attack. Assessment of naturally infected rice leaves is carried out at 30 days and 50 days after planting.

Table 1. Scoring of leaf blast and neck blast during rice mutant plants growing at hot spot area of blast disease.

Score	Leaf Blast		Neck Blast	
	30 DAS	**50 DAS**	**1st week**	**3rd week**
0	Mi-7D, Mi-22, Mi-28, Mi-82, Limboto	7D, Mi-22, Mi-28, Mi-82, Limboto	Mi-9, Mi-21, Mi-28, Mi-38, Mi- 39P,Mi-77, Limboto	Mi-28, Mi-77, Limboto
1	Mi-2, Mi-4, Mi-5, Mi-7, Mi-9,Mi-13, Mi-13P, Mi-14, Mi-16,Mi-17, Mi-21, Mi-29, Mi-29P,Mi-32, Mi-33, Mi-36P, Mi-37,Mi-38, Mi-39P, Mi-40, Mi-41,Mi-44P, Mi-45, Mi-46, Mi-49,Mi-50, Mi-51, Mi-52, Mi-59,Mi-68, Mi-70G, Mi-71, Mi-72, Mi-75, Mi-76, Mi-77, Mi-78,Mi-81, Mi-83, Mi-102, Mi-107, Mi-113, Mi-123, Mi-131, Mi- 142, Mi-145, Mi-151, Mi-153, Mi-155, Mi-158, Mi-167,Mi-181	Mi-4, Mi-7, Mi-9, Mi-13, Mi-13P, Mi-14, Mi-16, Mi-17, Mi-22,Mi-29, Mi-29P, Mi-32, Mi-33,Mi-37, Mi-38, Mi-40, Mi-42,Mi-44P, Mi-46, Mi-50, Mi-52,Mi-71, Mi-76, Mi-78, Mi-80,Mi-81, Mi-83, Mi-123, Mi-131, Mi144, Mi-145, Mi-151, Mi-153, Mi-158, Mi-341, Mi-361, Mi-391,Mi-181	Mi-4, Mi-13, Mi-14, Mi-22,Mi-29, Mi-29P, Mi-32, Mi-45, Mi-53, Mi-59, Mi-71, Mi-82, Mi-83, Mi-84, Mi-86, Mi-107, Mi-108, Mi-143, Mi-145,Mi-146, Mi-153, Mi-154, Mi-155, Mi-158, Mi-180	Mi-22, Mi-38, Mi-83, Mi180

3	Mi-9, Mi-34, Mi-36, Mi-39,Mi-42, Mi-46P, Mi-51, Mi-55, Mi-57, Mi-58P, Mi-60, Mi-62, Mi-63P, Mi-64P, Mi-66, Mi73, Mi-80, Mi-84, Mi-85,Mi-86, Mi-87, Mi-88, Mi-93, Mi-95, Mi-97, Mi-99, Mi-100, Mi-108, Mi-109, Mi-110, Mi-111,Mi-112, Mi-114,Mi-115,Mi-116, Mi-117,Mi-118, Mi-119,Mi-124,Mi-125, Mi-126,Mi-127, Mi-129,Mi-140,Mi-141, Mi-143,Mi-144, Mi-146, Mi-150, Mi-154, Mi-159, Mi-160, Mi-180, Mira-1	Mi-2, Mi-3, Mi-21, Mi-32, Mi-34,Mi-36P, Mi-39P, Mi-41, Mi-45,Mi-46P, Mi-49, Mi-51, Mi-57,Mi-59, Mi-63P, Mi-64P, Mi-66,Mi-68, Mi-70G, Mi-72, Mi-73, Mi-75, Mi-77, Mi-82, Mi-84, Mi-85, Mi-86, Mi-87, Mi-97, Mi-99,Mi-100, Mi-102, Mi-107, Mi-108, Mi-109, Mi-110, Mi-111, Mi-112, Mi-113, Mi-117, Mi-118, Mi-119, Mi-124, Mi-125, Mi-127, Mi-129, Mi-140, Mi-141, Mi-142, Mi-143, Mi-146, Mi-150, Mi-152, Mi-154, Mi-159, Mi-160, Mi-167,Mi-180, Mira-1	Mi-2, Mi-7D, Mi-13P, Mi-16,Mi-17, Mi-21, Mi-36P, Mi-37, Mi-40, Mi42, Mi-49, Mi-50, Mi52, Mi-64P, Mi-78, Mi85,Mi-88, Mi-131, Mi-144, Mi- 151, Mi-152, Mi-159, Mi-181	Mi-4, Mi-7D, Mi-9,Mi-13, Mi-13P, Mi-16, Mi-17,Mi-21, Mi-29, Mi-29P, Mi-32,Mi-36P, Mi-37, Mi-40, Mi-45, Mi-50,Mi-52, Mi-59, Mi-64P, Mi-76, Mi-78, Mi-82, Mi-131, Mi-158, Mi-181
5	Mi-99, Mi-138, IR64, Cisokan	Mi-39, Mi-55, Mi-58P, Mi-60,Mi-62, Mi-88, Mi-93, Mi-94,Mi-95, Mi-97, Mi-114, Mi-115, Mi-116, Mi-128, Mi-138, Cisokan	Mi-3, Mi-7, Mi-46, Mi-66,Mi-68,Mi-70G, Mi-73, Mi-73,Mi-75, Mi-78 Mi-79, Mi-80, Mi-81, Mi-87, Mi-94, Mi-90, Mi-100, Mi 102, Mi-109, Mi-110, Mi-111,Mi-119, Mi-123,Mi-124,Mi-125,Mi-138, Mi-140,Mi-141, Mi-142, Mi-143, Mi-150, Mi-160,Mira-1, Cisokan	Mi-2, Mi-3, Mi-7, Mi-9, Mi-14, Mi-29, MI-44P, Mi-46, Mi-49, Mi-66,Mi-68, Mi70G, Mi-71, Mi-72,Mi-73, Mi-75,Mi88,Mi-94,Mi-97,Mi-99, Mi-100, Mi-102, Mi-107, Mi-108, Mi- 109, Mi-111, Mi-112, Mi-119, Mi-123, Mi-124, Mi-125, Mi- 138, Mi-139, Mi-140, Mi-141, Mi-143, Mi-145, Mi-150, Mi- 151, Mi-152, Mi-153, Mi-154, Mi-155, Mi-159, Mi-167, Mi-180, Mira-1, Cisokan
7	Mi-44P, Mi-46P, Mi-51, Mi-55,Mi-57, Mi-58P,Mi-60, Mi-63P,Mi-97, Mi-112, Mi-113, Mi-114, Mi-115, Mi-116, Mi-126, Mi-127, Mi-167, IR64	IR64, Mi-58P, Mi-60, Mi-62, Mi-63P,Mi-80, Mi-81, Mi-85, Mi-86, Mi-87, Mi-93, Mi-110, Mi-113, Mi-114, Mi-115, Mi-116,Mi-117, Mi-118, Mi-126, Mi-127, Mi-128, Mi-129, Mi-146, Mi-160, IR64, Mira-1	Mi-33, Mi-39,	Mi-33, Mi-46P, Mi-55, Mi-57,
9	Mi-62, Mi-63, Mi-64	Mi-34, Mi-36, Mi-39, Mi-42, Mi-42, Mi-51, Mi-95		

Notes : 0 (Highly resistant = HR); 1 (Resistant = R); 3 (Moderately Resistant = MR); 5 (Moderately Susceptible = MS); 7 (Susceptible = S), 9 (Highly Susceptible = HS).

The variation of blast disease scores, based on a 1–9 scoring system on leaf damages, there were Mi-7D, Mi-22, Mi-28, and Mi-82 rice mutant lines highly resistant (HR) or 0 scores to leaf blast disease at 30 days and 50 days after sowing. Those lines are equal to the Limboto rice variety as a control of rice resistant to blast disease. Out of 47 rice mutant lines with resistant category, moderately resistant (MR) and moderately susceptible (MS) to leaf blast disease the scores of 1, 3, and 5 respectively. Mira-1 rice variety as a wild rice, shown moderately resistant (MR), however, Cisokan and IR64 rice varieties displayed susceptible character. The variation of the severity of rice mutant lines to leaf blast disease indicated that the genetic change occurred by gamma rays. This phenomenon is in line with Lagoda in Shu et.al, [19], who mentioned that irradiation of seeds disrupts protein synthesis, affects hormone balance, disturbs gas exchange, water exchange, and enzyme activity, and it will significantly affect physiological and biochemical processes in plants.

Blast disease can attack all parts of the rice plant including the neck or panicle side, causing empty seeds and decreasing of rice production. Out of 6 rice mutant lines displayed HR to neck blast disease namely Mi-9, Mi-21, Mi-28, Mi-38, Mi-39, and Mi-77 with a score of 0 respectively, equal to Limboto rice as a control resistant variety. About 25 rice mutant lines showed Resistant to neck blast disease with a score of 1 respectively. Another 23 rice mutant lines show MR character by the score of 3 at the beginning of the panicle until the generative stage. Mira-1 rice variety as a wild rice was moderately susceptible (MS) to neck blast disease. The previous study [22] found that rice mutant lines carry resistance to Xa5 bacterial leaf blast disease. Furthermore, Dwipa et.al [23] also reported from 17 brown and black rice tested, they found 7 genotypes were resistant and another 2 genotypes were moderately resistant to blast disease. Ulukapi et al [24] have developed resistance at varying frequencies to blast, bacterial blight, and tungoric disease using both chemicals and physical (fast neutron and gamma-ray) mutagens. Moreover, Kamolsukyeunyong et.al [25] obtained 16 mutant lines carrying the resistant allele of BPH32 and susceptible alleles of OsLecRK3 and OsSTPS2 from 9323 selected rice mutant lines screened. They also found substituting the susceptible A-G with the resistant G-T HPs, the damage-reducing ratios (DRRs) were 0.53, 0.46, and 0.25 against CNT, UBN, and TPY populations, respectively by SNP analysis. Li et al [10] mentioned that 17 genes of Lesion mimic mutant (LMM) are important members among DR genes that confer resistance against *M. oryzae* infection. Genetically, plant resistance can be divided into two, namely vertical resistance and horizontal resistance. Vertical resistance is resistance determined by one or a few genes or resistance to certain pathogenic lines, but not to other lines. Whereas horizontal resistance is plant resistance which is determined by many genes or host resistance to all pathogenic races [26].

Rianingsih et.al [27] reported that strains 001, 003, 033, and 173 *P.grisea* existed at Cikembar, Sukabumi village. Anggiani et al. [28] mentioned that strain 101 was the most dominant in irrigated rice by 24.6%, followed by 18.3 % and 11.4 % strains of 001 and 033 *P.grisea* respectively.

Figure 1 displays the performance of rice mutant lines in the hot spot area of Cikembar, Sukabumi. It was shown that rice mutant line (A) was very clean from infection of leaves blast disease compared to IR64 (B) as a susceptible rice variety. The panicle attack by neck blast is also displayed by the Mira-1 rice variety as a wild type (C) compared to the rice mutant line which has 3 scoring by neck blast disease.

Fig. 1. Performance of rice mutant lines in blast spot area (A. Mi-82 rice mutant line, B. IR64 rice variety, C. Mira-1 wild type rice, D. Mi-168 rice mutant line).

Mutation breeding is a very effective approach to developing new resistant genotypes to blast and bakanae disease in rice. We found that the percentage attacks of leaf and neck blast diseases on the Limboto rice variety was very low, shown at 1.50 % and 1.00 % respectively. This result means that Limboto was highly resistant to blast disease, and the same character was also found in the Mi-82 rice mutant line (Table 2).

Knowledge of the correlation between the resistance of plants in the field is very helpful for the selection of the plant's agronomic traits. The positive and significant relationship between the tiller number and with number of seeds per panicle indicates the importance of these traits in determining seed yield (Table 2).

Table 2. Agronomic characters of rice mutant lines at the Cikembar experiment field.

Rice lines	Leaf infection (%)	Neck infection (%)	TN	PL (cm)	GY	Weight of 1000 seeds (g)
Mira-1	20.57	32.36	14	24.4	132	25.73
Limboto	1.50	1.00	7	25	125	24.56
Mi-4	6.25	6.60	13	23.5	157	24.50
Mi-7D	4.50	9.80	12	26.5	112	26.10
Mi-9	5.22	8.41	17	27.2	156	27.93
Mi-13	5.00	8.65	13	22.8	145	26.24
Mi-13P	5.00	5.50	15	171	171	26.53
Mi-16	5.34	7.50	15	154	154	25.89
Mi-17	8.56	8.25	17	145	145	26.35
Mi-21	6.80	6.67	14	165	165	27.54
Mi-28	5.65	9.30	15	155	155	25.65
Mi-29P	4.49	6.00	19	157	157	26.13
Mi-29	4.49	6.00	19	157	157	26.13
Mi-32	6.02	7.53	19	148	148	25.37
Mi-37	4.45	6.64	21	152	152	26.97
Mi-38	4.45	9.65	15	145	145	27.32
Mi-40	3.25	3.50	16	123	123	26.75
Mi-150	8.53	8.41	15	276	153	27.30
Mi-152	7.87	4.48	15	25.00	120	26.50
Mi-176	8.50	7.50	18	25.00	135	26.86
Mi-177	7.60	7.87	16	26.90	134	27.12
Mi-182	2.35	4.50	16	28	168	26.13
Mi-183	3.53	4.67	17	26.90	146	27.80
Mi-181	5.45	6.25	13	24.80	143	27.10

Notes: Tiller Number (TN); P; Panicle Length (PL); Grain Yield per panicle (GY)

Table 2 displayed only the good performance of rice mutant lines obtained. Tillering in rice is an important agronomic trait for grain production. The highest tiller number was found in Mi-37, Mi-29P, and Mi-32 rice mutant lines respectively, and significantly different from their wild rice of Mira-1. The resistant trait of rice mutant lines was not linear with the agronomic characters, this means that mutation by gamma rays was a very random effect.

The panicle length plays a significant role in raising the yield per unit area of rice crop. Rice mutant lines showed a wide range of variability in the panicle length, ranging from 22.50 cm to 28.00 cm. Mi-82, Mi-9 and Mi-13P rice mutant lines were the longest panicle, and the panicle length of some rice mutant lines was shorter than their wild type. Naeem et.al [29] mentioned that radiation reduced the panicle length, thereby imparting a vigilant effect on KSK-133, reducing the PL up to 15.6 cm by 20 Krad irradiation of dose. The phenotypic coefficients of variability (15.05%) were slightly higher than those of the genotype (14.5%), which indicated that the variation was due to genetic content, with countable influence by the environment.

The number of grains per main panicle influences the grain weight, and it is an important trait influencing the yield and yield-contributing characters. Table 2 displayed that 171 filled grains per panicle had the highest grains found in the Mi-13P rice mutant line, followed by 168 grains per panicle in the Mi-82 rice mutant line. The variability of the number of filled grains per panicle in the rice mutant line shown was very high, there were ranging from 116 to 171. Haris et al. [30] reported that 200 Gy of radiation could produce variation through induced mutation. Wattoo et al. [31] observed a decrease in the number of grains per panicle as a result of an increase in gamma radiation from 15 Krad to 25 Krad; additionally, the mutant showed lesser number of grains per panicle compared to the non-radiated plant.

The grain weight per main panicle is the main characteristic of higher yield. Rice mutant line Mi-9 was the highest 1000 seeds of grain weight we obtained, followed by Mi-83, Mi-21, and Mi-181 which were 27.93 g, 27.80 g, 27.54 g, 27.32 g, and 27.10 g respectively. Mira-1 rice variety was the wild type of rice mutant line, it was only 25.90 g for 1000 seeds grain weight. Similar data was also obtained by mutation induction of Mentik susu rice by 200 Gy gamma-ray irradiation [32]. The treatment of gamma-ray irradiation on rice plants can affect the genotype of rice crops. Changes in plant genotypes can be seen from the measurement differences in each observed character observed. The irradiation carried out leads to an increase or even decrease in the observed character measured quantitatively.

4 Conclusion

The variation-resistant characters of rice mutant lines which were screened in the field of hot spot area of blast were found. It was 3.57 % of rice mutant lines highly resistant to leaf and neck blast diseases. The best agronomic characters were obtained in Mi-29, Mi-32, and Mi-82 rice mutant lines. These rice mutant lines were the best gene donors for blast rice resistant to blast disease.

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