Development Of New Type Upland Rice Lines For Resistance To Blast Disease Through Anther Culture

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ABSTRACT

The blast disease caused by *Magnaporthe oryzae* is an important disease on upland rice due to its rapid spreading rate andquite high to the host. One effort to overcome the obstacle is to use resistant varieties to the disease. Anther culture is one of tissue culture methods which can be applied to plant breeding programs in order to accelerate the process of obtaining pure lines. The successful development of rice varieties is highly dependent on genetic diversity and desirable traits. To obtain the genetic variability of doubled haploid lines through anther culture techniques, anther F_1 or F_2 were used as explants sources.The objective of the study were to develope of new plant type of upland rice through anther culture and to evaluate the resistance of the obtained double haploids(DH) to blast desease. Anthers from the F_1plants of the crosses between Fatmawati and varieties of resistance to the bast disease were cultured and 348 DH lines were obtained. However, only 58 DH lines were suitable for upland rice development. After inoculating three isolates (race 173, 033, and 001) of *M. oryzae*, 26 DHlines showed resistance to all of the isolates. Lines P3-27, P5-50, P6-105, P3-162, and P3-204 possessed high yield potential with new plant type (more than six tillers), and resistance to blast desease.
Key word: New type, upland rice, anther culture, blast resistance

INTRODUCTION

The contribution of upland rice to the rice production in Indonesia is still relatively low where the productivity of upland rice amounted to 2.95 ton/ha, much lower than the

productivity of lowland rice (5.15 tons/ha) (Indonesian Department of Agriculture, 2009). The low productivity of upland rice is mainly caused by variations in climatic and soil conditions, poor cultivation methods in the terms of varieties, fertilizers and the control of the blast disease caused by *Magnaporthe oryzae*. The desirable traits of upland rice for the local conditions is the short to medium mature periods, medium tillering ability, slightly upright stem, resistance to the blast disease and tolerance of aluminum damage, drought and shading (Lubis *et al*., 1993).

The blast disease is an important disease on upland rice crops due to the rapid rate of spread and damage caused quite high. One effort to overcome these obstacles is to use resistant varieties/tolerant of the existing constraints.

Anther culture is a tissue culture technique which can be applied in plant breeding to accelerate the process of obtaining pure lines. The success of the New Plant Type (NPT) engineering depends on the genetic variability and the desired characters. High genetic variabilities in a single cross occurred on the second (F2) and third (F3) generation, and the F2 have all combinations of genes in the expected genotypes. Combinations of characters derived from the two parental lines occurred in the haploid plants, so if the chromosomes were doubled, or spontaneous doubling occurred during the culture, a double haploid or dihaploid (DH) pure lines will be obtained. Selection for the desired characters can be done earlier on the DH1 or DH2, hence will take less time compared to a conventional breeding.

Superior rice varieties with high yields are expected to support self-sufficiency food program in Indonesia. International Rice Research Institute (IRRI) have formulated prototypes of NPT of rice in 1989 (IRRI, 1990). The desired characters of the NPT rice are: the compact growth, the number of productive tillers of 8-10, the big panicles and good grain filling (200-250 grains), the semi dwarf (80-100 cm in height), the erect and thick leaves with dark green colour, the medium earliness (100-130 days), the deep rooting system, and the high resistance to major pests and diseases (Khush, 1995). Developing new types of upland rice has not been done considering the constraints of environmental adaptation and biotic stress. The development of new type of upland rice required a modified properties of a new type of lowland rice , among others are: the heavy panicle (> 150 grains per panicle), all of productive tillers (> 6), the plant height less than 150 cm, an early maturity (less than 120 days), the flag leaf angle 5^{0}-10^{0}, and tolerance to aluminium and blast disease resistance (Herawati, 2009).

There have been limited efforts to produce NPT of upland rice due to environmental constraints and biotic stresses. The aim of this study was to obtain a new plant type of upland rice through anther cultures and to evaluate the obtained double haploid lines that posseses the blast disease resistant.

MATERIALS AND METHODS

Anther cultures of rice plants

The research was conducted in the Laboratory and greenhouse of the Research and Development Center for Biotechnology and Genetic Resources for Agriculture in Bogor (West Java, Indonesia); the rice varieties employed were: Fatmawati which has the properties of new types, Way Rarem, SGJT-36, and SGJT-28, that are rice varieties resistant to blast disease (Bakhtiar, 2007). Anther crosses of rice plants (F1) consisting of selected lines of P1 (Fatmawati x Way Rarem), P2 (Fatmawati x SGJT -28), P3 (Fatmawati x SGJT-36), P4 (Way Rarem x Fatmawati), P5 (SGJT-28 x Fatmawati), and P6 (SGJT-36 x Fatmawati), callus induction media (N6), and the regeneration medium (Murashige Skooge) (Dewi, 2003).

The basic medium for callus induction (N6 medium) has 2.0 mg/L of NAA (Naphthalene Acetic Acid) and 0.5 mg/L of Kinetin. The regeneration and rooting medium (MS medium) has 0.5 mg/L of NAA and 2.0 mg/L of Kinetin. Each petri dish containing of 125-150 anthers from 25-30 rice flowers (spikelet). Callus derived from cultures of pollen in the anthers were incubated in the dark room temperature 25 ± 2 ° C (Li, 1992). The compact calluses obtained of 1-2 mm in size were directly transferred into culture bottles that already contain 25 mL of regeneration media. Green plants that grow from the callus on regeneration medium and reach a height of 3-5 cm (after approximately one week) were transferred into culture tubes containing 15 mL of rooting medium. Experiments using Completely Randomized Design with 15 time replicated. Observations were done on the number of anthers inoculated, the amount of callus formed, the number of calli that produced plants (albino and green), the total number of plants (green + albino), the number of green plants, the number of albino plants, and the number of spontaneous double haploid plants (plants fertile).

Screening of Double haploid Lines of New Type of Upland Rice Obtained from Anther Cultures Resistant to the Blast Disease

The testing of resistance of DH rice plants to blast disease was done in the house blast incubation on the Indonesia Rice Research Institute in Muara, Bogor (West Java, Indonesia). Artificial inoculation was done using isolate dominant races in accordance with the purpose of breeding, following the method of Utami et al. (2000). The plant selected were 58 double haploid lines (DH): four parents were SGJT-28, SGJT-36, Way Rarem, and Fatmawati, and Kencana Bali rice cultivars as susceptible controls and Asahan as resistant controls. Test isolates representing three major groups Pyricularia grisea Sacc haplotype: Group I represents the race avirulent, widespread and able to survive long in the field represented by the 001 race, the Group II that represents an extremely varied racial virulence and is thought to be able to adapt to selection pressure represented by the 033 race and the Group III represented by a very virulent race, but did not survive long in the field and are represented by races 173(Utami et al., 2000). Media for propagation of isolates was potato dextrose agar (PDA) and oat meal agar (OMA). 20seedsselectedat eachgenotype in the oven for 3 to 24 hours at a temperature of 45°C and then planted in rows with spacing of 3 x 4 cm in plastic tubes (38 cm x 26 cm x 10 cm) filled with soil from the garden experiment BB-Biogen Ceukemeuh in Bogor. Inoculationwas done bysprayinginoculumof 50ml/plastic tubesonthe phaseof seedlings(at3-4leafstadia or 18 days after planting).14 genotypes were tested with four parents and a control of resistant and sensitive. The experiments were analyzed at 7 day after planting (DAP) and qualify the disease scale with a scoring system based on the Standard Evaluation for Blast Disease (IRRI, 1996).

Evaluation of Agronomy Character of the New Type of Double Haploid Upland Rice Lines Obtained from the Anther Cultures in the Field

These experiments were conducted at the Experimental Garden Babakan, Dramaga, Bogor in the Agricultural University, West Java, Indonesia. A total of 20 lines of upland rice derivate from anther culture and screening blast disease and two varieties as controls namely Fatmawati and Way Rarem and two lines: SGJT28, and SGJT-36 were employed in these experiments. Plots measuring 4 x 4 m with 16 rows, in each genotype were planted two rows and in each row there were 14 holes and were planted two seeds per hole. Plant spacing between rows 20 cm x 30 cm and 40 cm of spacing between lines. The experimental using Augmented Design without replicated.

The mean adjusted of double haploid genotypes that were tested were obtained after the block effect was calculated. Least Significant Increase (LSI) between the average value of adjusted each character a genotype test with an average of one genotype control (Baihaki, 2000). Observations were made in eight clusters which represent every line in random order, namely plant height, date of maturity, number of productive tillers, number of total grain/panicle, percentage of grain fill/panicle, and weight of grain/hill.

RESULTS

Anther culture of rice plants

The rice crosses showed significant results on plant regeneration; indicated by the variable number of green plants and the number of albino plants obtained. The total number of green plants produced by crossing the most P3 (Fatmawati x SGJT-36) was 1.14 to 39.2 %, P6 (SGJT-36 x Fatmawati) produced a 10.3 to 37.6% (Table 1, Figure 1).

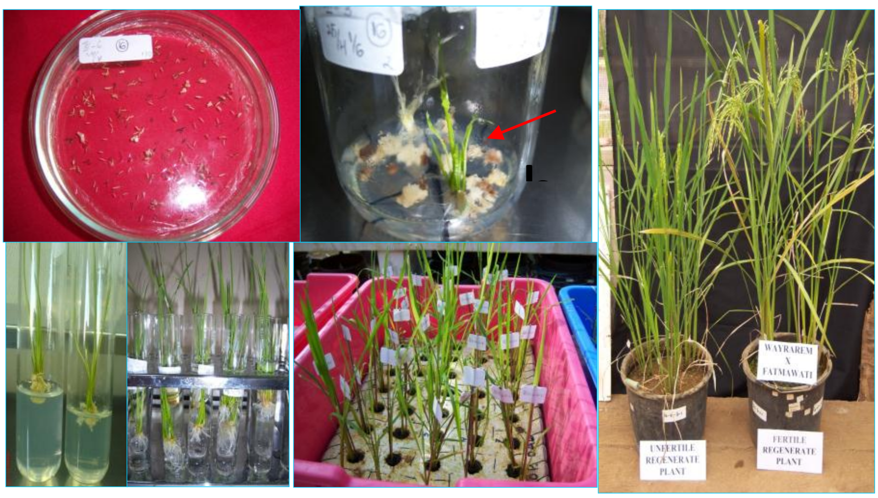

Figure 1. Anther culture process to develope new type of upland rice

Table 1.Crossing response from anther cultures of rice plants regenerated

The origin of crosses	Plant Regenerated[1)]		Culture efficiency[2)]		Number of		
	Green	Albino	Ratio of GP to RC	Persent of GP to Anther	GP[*)] accli-mated	PP	Doubled Haploid lines
P1 (Fatmawati /W.Rarem)	1.5(22.5)	11.9(77.5)	0.6b	1.0cd	13	13	6(46.2)
P2 (Fatmawati /SGJT-28)	1.6 (24.9)	10.6(75.1)	0.4b	1.1c	22	22	13(59.1)
P3 (Fatmawati/ SGJT-36)	14.1(39.2)	26.7(60.8)	1.0a	10.7a	387	364	187(51.4)
P4 (W.Rarem /Fatmawati)	0.2(6.7)	4.7(93.3)	0.2b	0.2d	3	3	3(100)
P5 (SGJT-28/ Fatmawati)	0.6 (11.2)	6.1(88.8)	0.3b	0.5cd	9	7	5(71.4)
P6 (SGJT-36 /Fatmawati)	10.3(37.6)	19.1(62.4)	1.0a	8.4b	252	242	134(55.4)
Total					686	651	348(53.5)

[1,2)]means of 15 petridish,[*)]GP =green plantlets;RC=Responding calli; PP=plants are planted; the number of ( ) is percentage

The highest number of albino plants produced was from the crossing of P3 (Fatmawati x SGJT-36): 7.26 to 60.8%, followed by crosses P6 (SGJT-36 x Fatmawati) with 1.19 to 62.4%. The crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) of the ratio of green plants from calli to produced plant was 1.0, and percent of total anther green plants are inoculated each at 7.10 and 8.4% (Table 1). This shows that both combinations crosses efficiency in producing green plantlets better than the other crosses. This research is produced the percentage of double haploid green plants amounted was 53.5% of the total green plants regenerated; of all the plants that were acclimatized 88.3% of the 777 plantlets; the 94.9% of the 686 acclimatized plants were planted and selected a 348 double haploid (fertile) plants (53.5% of 651 plants planted). Select double haploid lines were: 6 double haploid lines from Fatmawati/Way Rarem (P1), 13 double haploid lines from Fatmawati/SGJT-28 (P2), 187 double haploid lines from Fatmawati/SGJT-36 (P3), 3 double haploid lines from Way Rarem/Fatmawati (P4), 5 double haploid lines from SGJT-28/Fatmawati (P5) and 134 double haploid lines from SGJT-36/Fatmawati (P6) ready to be evaluated further.

Screening of Double Haploid (DH) Lines for Their Resistance to Blast Disease

The results showed that the genotypes that were tested gave varied responses to the three races of *P. grisea* used. Screening of leaf blast on the 58 lines showed 26 double haploid lines resistant to all races (173, 033, and 001), one double haploid lines resistant to races 033 and 173, one double haploid lines resistant to races 001 and 173, 15 double haploid lines resistant to races 033 and 001. There was only resistant to one race. 5 double haploid lines resistant to race 001 and 2 double haploid lines resistant to race 033 (Table 2; Figure 2).

Table 2.Screening of leaf blast on double haploid lines of a new type of upland rice obtained from the anther cultures

Race of blast	Number of resistant lines[*)]	Genotipe
173, 033, and 001	26	P3-27, P6-278, P3-26, P6-319, P6-320, P6-311, P6-317, P3-161, P6-267, P4-45, P6-291, P3-28, P3-175, P6-286, P6-314, P6-266,P3-160, P3-162, P3-250, P6-297, P3-249, P6-262, P3-31, P6-264, P3-196, P6-302
173 and 033	1	P6-295
173 and 001	1	P6-276
033 and 001	15	P3-150, P3-191, P3-210, P3-221, P6-265, P3-238, P3-204, P3-248, P3-190, P6-62, P3-120, P3-131, P3-135, P3-148, P3-158
173	0	-
033	2	P6-105, P3-134
001	5	P6-275, P6-273, P6-50, P6-255, P3-159

Note :[*)] Leaf blast: highly resistant (0%), resistant (<10%), susceptible (>10 %)

Figure 2. Screening of doubled haploid lines of new type upland rice derivated anther culture for resistance to blast disease

Evaluation of the Agronomy Character of the Double Haploid Lines Obtained from Anther Cultures n the Upland

Twenty (20) double haploid lines had high yield potential, these were: P3-27, P5-50, P6-105, P3-162, and P3-204 with a weight of grains/hill more than 30g. Lines: P3-28, P4-45, P3-134, and P3-175, had more than 20 g/ hill; while other lines of low potential were less than 20 g/hill (Table 3). Almost all lines tested have maturity ranging between 101 to 127 days; but plant height varies between 81.17 to167.5 cm. Some lines had the important characters of NPT among others; almost of all had a productive tiller number (> 6) with heavy panicles (grain fill > 150 grains/panicle) (Table 3).

Table 3. Traits or characteristics of the 20 double haploid lines of upland rice in dry land

	Characters							Race of leaf blast		
Line	WG*	Yield (ton/ha)	PT	NTG	%GF	PH	M	173	033	001
P2-1	13.56	2.26	11.33	264.11	38.54	81.17	110	MS	MS	MS
P2-2	13.00	2.17	11.17	301.78	30.06	87.83	110	MS	MS	MS
P3-27	31.88	5.31	6.83	397.44	52.01	160.17	115	R	R	R
P3-28	26.42	4.40	7.17	423.67	42.15	162.17	115	R	R	R
P3-31	13.51	2.25	9.00	223.17	47.30	114.83	123	R	R	R
P4-45	26.21	4.37	9.17	256.22	67.91	124.33	110	R	R	R
P5-50	37.92	6.32	13.00	214.39	85.31	127.83	110	S	MS	R
P6-62	10.39	1.73	6.40	342.78	23.14	125.67	110	MS	S	R
P6-103	8.27	1.38	11.17	265.17	28.87	149.50	127	S	S	MS
P6-105	41.53	6.92	12.33	357.33	51.62	159.83	110	S	S	MS
P1-108	10.56	1.76	11.50	299.78	28.34	113.17	101	MS	MR	R
P3-120	6.32	1.05	5.67	359.50	22.70	142.00	115	S	R	R
P3-131	9.92	1.65	8.60	335.56	25.70	150.33	110	S	R	R
P3-134	29.93	4.99	11.50	221.72	51.87	140.50	110	S	R	MS
P3-135	8.15	1.36	9.33	214.06	26.45	167.50	115	S	R	R
P3-150	6.74	1.12	10.00	232.28	17.20	149.83	115	S	MR	R
P3-162	41.44	6.91	9.83	434.61	55.34	154.67	110	R	R	R
P3-175	23.44	3.91	12.50	171.28	56.44	99.17	110	R	R	R
P3-204	31.03	5.17	9.50	307.94	52.14	151.50	101	MS	R	R
P6-319	4.01	0.67	11.33	333.06	10.73	153.33	115	S	R	R
Fatmawati	24.20	4.03	9.97	302.17	47.48	108.97	98.6	MS	MS	MS
W. Rarem	30.93	5.16	13.30	283.72	60.28	131.00	101.6	MS	R	MR

*WG=weight grains/hill, PT=productive tillers, NTG=number of total grain/panicle, %GF=% grains fill/panicle, PH=plant hight, M=maturity, MS=moderate susceptible, S=susceptible, MR=moderate resistant, R=resistant

RESULTS AND DISCUSSION

The high frequency of double haploid plants generated in this study due to genetic factors and the high success of the acclimatization. Treatments during the pre-culture treatment of low temperature

(5 °C) and the addition of10-3M put rescine to the media in this experiment in creased callus induction and green plant regeneration in anther culture of rice plants. Pre-treatment of low temperatures will homogenize the pollen on the stadia uninucleid thus enhancing the induction of embryo genesis produce green plants, while the polyamine has the function of inhibiting senescence on anther culture through inhibition of the biosynthesis of ethylene, so that the viability of the cell wall of the tapetum can be maintained longer in support of and rogenesis (Dewi, 2003; Purwoko et al., 2001; Dewi et al., 2007). The crossing P3 and P6 has the capability of anther culture, which is quite high. According to Li (1992) used crosses or reciprocal crosses will cause the alteration in power anther culture (anther culture ability), and expression of a trait, so it was found that the sequence of the rice subspecies ability to produce green plants in anther culture is a Japonica/indica>japonica>indica. At the crossing of P3 (Fatmawati x SGJT-36) and P6 (SGJT-36 x Fatmawati) ratio of green plants to produced plant callus of 1.0, and percent of total anther green plants are inoculated each at 7.10 and 8.4 percent (Table 1). This shows that both combinations crosses efficiency in producing green plantlets better than the other crosses. combination of P3 and P6 crosses in this study have a great chance in the selection process to obtain the desired character.

This research was produced 348 green plants (53.5 percent of the total green plants regenerated) that can be used for further selection of materials. After selection for a new type of upland rice produced 58 doubled haploid lines. This material is used for selection of blast disease and the evaluation of the high yield.

In general, lines that are resistant to leaf blast in this experiment. According Fast selection method in stadia seedlings in this experimental screening doubled haploid lines of leaf blast resistance carried out in greenhouse using artificial isolates (race 173, race 033 and race 001) is very efficient in the early stages of plant breeding with a relatively small amount of seed before being tested in the field.

In this study, leaf spot symptoms on the susceptible genotype appeared at 3 to 4 days after inoculation (DAI). These spots continue to develop into large patches, a rhombus-shaped with the middle gray to white. Wang et al. (2007) reported that it takes twenty-four hours by P. grisea to induce spotting on the rice and 48 hours to activate defense responses in resistant genotypes. Left turn on gene expression of DR (defense related gene) along with the possibility of spore penetration time of blast in rice. Some genotypes that were tested showed no blast disease attack symptoms until 7 days after inoculation. The genotypes did not show symptoms until the end of the observation because these genotypes have the ability to limit the penetration apresorium blast.

Stratified by race, there are 25 genotypes resistant and 3 genotypes moderate resistance to race 173, 44 genotypes resistant and 3 genotypes moderate resistance to race 033, and 49 genotypes resistant to race 001 (data not shown). The number of genotypes that are resistant to race 001 more than the number of genotypes that are resistant to race 173 and race 033, because 001 is less virulent than race 173 and race 033 race. Race 001 has very low virulence, but its spread wide and able to survive long in the field, race 003 has a very diverse levels of virulence and able to adapt to selection pressures and have high virulence of race 173 but did not survive long in the field. The intensity of the attack to describe the magnitude of the disease attack rate in the population of certain genotypes.The higher level of leaf damage with increased intensity of the attacks. In general, the genotype that has an intensity of attack less than 10% showed a longer latent period and low disease scale (data not shown).

Evaluation of 20 doubled haploid lines obtained 5 lines have high yield, ie. P3-27, P5-50, P6-105, P3-162, and P3-204. These lines have weight of grain per hill more than 30 grams, it's equivalent with a yield more than 5 tons/hectare. The lines on the average have a plant high is more than 150 cm except for line P5-50. P5-50 line has a plant height desired for new types of upland rice is 127.83 cm. Lines that have a height of more than 150 cm is logging very vulnerable, and often found in a local upland rice varieties (Barus, 2008). These lines could be improved by crossed in the short lines to obtain moderate plant high (120-130 cm) that are not easily logging on the ground.

In terms of productive tillers, five lines had a number of productive tillers more than 6. Productive tillers is one component of which directly influence the high and low grain yield. The new paradigm of a new type of rice breeding is the number of productive tillers between 8-12 stems /hill by the number of grain/panicle ranged from 150-200 grains (Peng and Khush 2003). New Plant Type of rice have 20-25 panicles, but only 14-15 panicles can be harvested, with the number of rice grains per panicle 100-130. This is due to tillers that grow and late maturity can not be harvested. Tillers that are not productive is a competitor of productive tillers to get sunlight and nutrients. Criteria for a new type of upland rice is>6 productive tillers to support high yields in dry land. This is consistent with that suggested by Peng et al. (1994) that the capacity is low to the tillers of New Plant Type of rice are 3-4 productive tillers for a direct planting system (seeded), and 8-10 tillers for transplanted seedling (transplanting).

From the yield components, five of the lines have long panicles (> 27 cm) and thick (> 200 grains/ panicle), but grain filling about 50 percent except in line of P5-50 (grain filling> 85 percent). However, these lines have an average amount of grain filled more than 150 grains/panicle. Almost all lines tested have a short maturity ranged between 101-115 days. Local upland rice generally have a maturity more than 150 days. In the tropics, the optimum maturity of varieties to be potentially high yield is 120 days. Shorter life typically have a low potential because plants do not have enough time to use sunlight and nutrients in the soil, so it does not have enough time for vegetative growth for maximum yield (Yoshida and Parao 1976). Therefore, the age of 100-130 days varieties of rice are expected to give a yield as expected (Abdullah, 2005).

All lines are selected to meet the criteria important properties of new type upland rice specially in dense of panicles (> 150 grain content / panicle) and the number of productive tillers (> 6), with moderate maturity, but the lines still have weaknesses in plant height (> 150 cm), and the percentage of grains filling (51.6-55.3 percent), except line P5-50 (grain filling 85.3 percent).

All lines tested had different levels of resistance to blast disease. The selected lines should be further evaluated to determine the stability of the characters in each generation, especially for testing the results in the field.

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