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The inheritance AT *Xa27* Gene in F_2 progenies of Rice crossed between ting gong and IRBB27

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Abstract

Ting gong is a local rice variety that is famous for its fluffy rice texture and good adaptability. Recently this variety rarely cultivated by farmers because its deep-aged and susceptible to bacterial bligh disease. Improved performance of this variety can be done through plant breeding programs needs to be carried out. One method that can be done is crossing by utilizing the introduced variety as the source of the target gene. This study aims to analyze F_2 plants that carry the *Xa27* gene and evaluate their agronomic aspects. The study was conducted in a screen house, Plant Disease Laboratory and Plant Breeding Laboratory, Faculty of Agriculture, Syiah Kuala University, from July 2017 to January 2018. The seeds used were F_2 progenies from a crossing between IRBB27 and local variety Ting gong. Each pot is planted with two plants and there were 12 individual plants for analysis. Resistance test for *Xanthomonas oryzae pv. oryzae* and molecular analysis were carried out to identify the presence of target genes (*Xa27*) in F_2 plants. The results showed that 91.66% of the plants showed resistant symptoms (R) against *Xanthomonas oryzae* pv. *oryzae*, and 8.3% plant shows mildly susceptible to bacterial blight (MS).

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Introduction

Increased rice production has often been constrained by biotic stresses, one of which is bacterial leaf blight (BLB) caused by *Xhantomonas oryzae* pv. *oryzae* (Xoo). BLB is one of the most serious diseases in rice-producing regions in the world, especially Asia (Gnanamanickam *et al.*, 1999). The loss of yield of rice plants caused by BLB can reach 20-30% even in severe cases up to 50% in several regions in Asia (Mew *et al.*, 1993).

Utilization of resistance genes (R) is the most efficient method for controlling this disease. In particular, six R-genes are *Xa1, Xa5, Xa13, Xa21, Xa26* and *Xa27* have been obtained through genetic map-based cloning (Sun *et al.*, 2004).

In addition, the *Xa27* locus also provides high levels of resistance to 27 strains and moderate resistance to three strains of Xoo. The *Xa27* locus has been used and developed by plant breeders so that it has produced IRBB27 which shows semi-dominance in its genetic traits.

This disease will arise and develop quickly at high humidity levels. Therefore, Xoo often arises in plants with continuous spacing and irrigation (Sudir and Kadir, 2009). In conditions of tight spacing, it will be easier to infect plants from sick plants to healthy plants due to friction supported by strong winds and rain. This condition has an important role in the transmission and spread of pathogens (Suparyono *et al.*, 2016).

Leaf bacterial blight can attack plants in various phases starting from seeds, vegetative to generative phases. Of the many Xanthomonas groups, pathotype VIII is a pathotype that has a high virulence level and is spread in South Sulawesi, West Java, Central Java, East Java, Bali and North Sumatra (Sudir *et al.*, 2015). The use of resistant varieties is one method that has been considered the most effective and does not cause side effects such as the use of pesticides. Assembling resistant varieties is very dependent on available genetic resources.

Genetic resources in plant breeding can be utilized from germplasm and introduction. Local varieties with superior properties need to be conserved as assets of genetic resources that can be utilized in plant breeding programs (Sitaresmi *et al.*, 2013). However, this method is constrained by the ability of pathogens to form new virulent pathotypes so that plants that were previously resistant become sensitive (Suparyono *et al.*, 2016). Therefore, the development of resistant varieties must continue and planting varieties must be adapted to existing or specific path types in an area.

The insertion of the *Xa27* gene into Ting gong is very necessary because this variety is susceptible to BLB disease. It is expected that individuals plant that inherit the *Xa27* gene can show resistance to BLB and has good agronomic character.

Based on the above problems, it is necessary to conduct molecular analysis of the presence of *Xa27* gene in F_2 plants and evaluate agronomic characters and their level of resistance to leaf VIII bacterial leaf blight, in order to obtain resistant and high-yielding individuals.

Materials and methods

Plant Cultivation

This research was conducted in screen house, Plant Disease Laboratory and Plant Breeding Laboratory, Faculty of Agriculture, Syiah Kuala University. This research started from July 2017 until January 2018. The planting material used was the F_2 progenies of the plant which resulted from crossing between Ting gong and IRBB27. Each pot is planted with 2 plants so that there were 12 individual plants that were using for the analysis.

The seeds are first soaked for 24 hours and continued for 24 hours for germination process by using a wet towel. The seeds were sown on preparec media and transferred to pots at the age of 15 days.

The planting medium uses a mixture of soil and manure in a ratio of 2: 1. The first fertilization was

carried out the day before transplanting using 2.6 g of NPK fertilizer and 0.6 g of urea. Supplementary fertilization was carried out at the age of 2 and 5 weeks after planting (MST) using 0.6 g of urea fertilizer.

Molecular Markers

The molecular marker for the *Xa27* gene is a co-dominant *RMXa27*, located 35 kb upstream of the *Xa27* gene with oligonucleatide. (F: 5-ACGCTCCACCTACGCATGTCCT-3; R: 5-TCACACGGATTTTGAATGGTTCGGA-3) (Luo and Yin, 2013).

PCR amplification of molecular markers was performed on a PTC-100 programmable thermal control-ler (MJ Research, USA). The PCR reaction mixture of 10 µL consisted of 1 µL of rice genomic DNA, 0.10 µL of each primer, 5 ml Emirald Amp and 3.8 µL water (TaKaRa BIO INC, Japan).

Template DNA was initially denatured at 94 ^{o}C for 2 min followed by 35 cycles of PCR amplification with the following parameters: 30 s of denaturation at 94 ^{o}C, 40 s of primer annealing at 60 ^{o}C for markers *Xa27*, and 1 min to 2 min of primer extension at 72 ^{o}C according to the marker fragment length. Finally, the reaction mixture was maintained at 72 ^{o}C for 5 min before completion. The amplified products were electrophoretically resolved on a 1.5 % agarose gel for markers *RMXa27* in 19 TAE buffer.

Bacterial Inoculation and Scoring

The bacterial isolation was done at the Plant Disease Science Laboratory, Faculty of Agriculture, Syiah Kuala University. Inoculum bacteria *Xanthomonas oryzae* pv. *oryzae* (Xoo) used was Patotype VIII obtained from Indonesian Center for Rice Research of Muara, Bogor. Xoo was grown on PDA medium (peptone 10 g / L, sucrose 10 g / L, glutamic acid 1 g / L, 16 b / g bakto-agar, pH 7.0) for 2 days at 28 ° C. Bacterial cells suspended in sterile water at optical density of 0.5 (OD600) (Luo *et al.*, 2012). Xoo inoculation was performed using leaf clipping method (Kauffman, 1973), by cutting the tip of each plant (starting 3 leaves on each tillers of 5 leaves) with scissors dipped in Xoo suspension. The process of infection was done in the afternoon to avoid the heat and high evaporation. The length of the lesion (LL) was measured 1 week after inoculation up to week 3. Symptoms of the diseases were assessed to be resistant if (LL ≤ 3 cm), moderately resistant (LL> 3 cm, ≤ 6 cm), moderately susceptible(LL> 6 cm, ≤ 9 cm) or susceptible (LL> 9 cm) (Amante-Bordeos *et al.*, 1992).

Phenotypic and Agronomic Observation

Phenotypic observation for bacterial blight resistant was performed by recorded the lesion lenght at 7, 14 and 21 days after bacterial inoculation (DAI). To analyze agronomic character of the progenies. The variables observed included the number of productive tillers, panicle length, grain weight, 1000 grain weight, percentage of filled grain, empty grain percentage and yield potential h^{-1}.

Results and discussion

Resistance Evaluation to Bacterial Leaf Blight

The results showed that there were differences in the level of resistance of 12 plants tested against BLB strain VIII. Sensitive plants show symptoms of wilting and the color of the leaves begins to turn yellow especially in the area of infection / cutting. At 7 and 14 days after inoculation (DAI) showed that all plants had not shown symptoms of bacterial leaf blight deseases. However after observations at 21 days after inoculation (DAI) showed that 91.66% of plants were resistant to Xoo strain VIII and 8.3% of plants showed mildly susceptible symptoms (MS) (Table 1, Fig. 1).

Plant response to BLB deseases varies greatly in each plant every week. The spread of bacterial leaf blight is very dependent on climatic conditions. Low humidity levels and far spacing will slow the rate of infection and spread of Xoo. Based on the results of molecular analysis (Fig. 2) it can be seen that of the 12 individual plants there were 3 (25%) plants that did not inherit the *Xa27* gene, namely T14, T19, and T22. 9 (75%) F_2 derivatives molecularly inherited the *Xa27*

gene and were agronomically proven to be resistant to Xoo strain VIII infection (Fig 2, Table 1).

T14 individuals molecularly do not inherit the Xa27 gene and have been shown to be somewhat susceptible when tested with Xoo strain VIII.It is suspected that differences in the expression and non-expression resistance properties are caused by differences in genetic composition in various plants. In addition, differences in genetic composition will affect the response of plants to the environment because the growing place of rice plants gives effect to environmental variations that affect phenotypic character differences in their genetic composition (Nusifera *et al*., 2014).

Table 1. The average length of lesions after inoculation with Xoo strain VIII F_2 progenies of rice.

Parental Lines	Xoo Strain VIII					
	Weeks 1		Weeks 2		Weeks 3	
	Lesion Length (cm)		Lesion Length (cm)		Lesion Length (cm)	
Tg/IRBB27 T_{13}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,00 ± 0,00	R
Tg/IRBB27 T_{14}	0,20 ± 0,20	R	0,20 ± 0,20	R	8,40 ± 7,67	MS
Tg/IRBB27 T_{15}	0,00 ± 0,00	R	0,00 ± 0,00	R	1,70 ± 1,11	R
Tg/IRBB27 T_{16}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,10 ± 0,10	R
Tg/IRBB27 T_{17}	0,10 ± 0,10	R	0,10 ± 0,10	R	0,20 ± 0,12	R
Tg/IRBB27 T_{18}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,00 ± 0,00	R
Tg/IRBB27 T_{19}	0,30 ± 0,30	R	0,40 ± 0,29	R	0,30 ± 0,20	R
Tg/IRBB27 T_{20}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,50 ± 0,39	R
Tg/IRBB27 T_{21}	1,90 ± 1,90	R	2,00 ± 2,00	R	2,60 ± 2,60	R
Tg/IRBB27 T_{22}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,40 ± 0,40	R
Tg/IRBB27 T_{23}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,00 ± 0,00	R
Tg/IRBB27 T_{24}	0,00 ± 0,00	R	0,00 ± 0,00	R	0,00 ± 0,00	R

Description: R= resistant (lesion length ≤3,0 cm), MR= moderately resistant (3,0 cm <lesion length ≤6,0 cm), MS= moderately susceptible (6,0 cm<lesion length ≤9,0 cm), S susceptible (lesion length >9,0 cm).

According to Abadi (2003) the diversity of resistance/sensitivity reactions to pathogens among plant varieties is caused by different resistance genes in each variety. Gen *Xa27* is one of the genes that can suppress the development of Xoo and is dominant (Ogawa, 1993). Resistance properties are controlled by one or two genes more than the dominant genes (Ou, 1985).

Table 2. Potential yields of F_2 progenies of and its comparison with their parents IRBB27 and Ting gong.

Parental Lines	NPT	PL	Grain Weight	1000 Granule	% Pithy Grain	% Empty Grain	Potential yield (ton h^{-1})
Ting gong	9,75	25,77	46,34	20,21	91,78	8,22	11,58
IRBB	8,88	22,38	24,26	23,55	57,81	42,19	6,06
Tg/IRBB27 T_{13}	6,00	28,33	42,09	22,30	89,00	11,00	10,52
Tg/IRBB27 T_{14}	6,00	25,00	17,08	22,70	61,49	38,51	4,27
Tg/IRBB27 T_{15}	7,00	21,14	24,51	21,00	90,83	9,17	6,13
Tg/IRBB27 T_{16}	10,00	24,00	39,55	22,40	91,37	8,63	9,89
Tg/IRBB27 T_{17}	9,00	24,78	33,51	21,00	91,47	8,53	8,38
Tg/IRBB27 T_{18}	10,00	23,10	36,55	21,10	91,45	8,55	9,14
Tg/IRBB27 T_{19}	11,00	22,64	32,45	23,80	66,89	33,11	8,11
Tg/IRBB27 T_{20}	8,00	26,13	35,43	24,70	88,19	11,81	8,86
Tg/IRBB27 T_{21}	11,00	23,36	36,07	21,50	80,75	19,25	9,02
Tg/IRBB27 T_{22}	12,00	23,92	36,17	25,90	82,32	17,68	9,04
Tg/IRBB27 T_{23}	10,00	27,40	55,05	22,70	86,97	13,03	13,76
Tg/IRBB27 T_{24}	11,00	23,09	32,13	21,80	87,95	12,05	8,03
Average	9,31	24,27	34,83	22,34	84,56	15,44	8,71

Description: number productive tillers (NPT), panicle length (PL).

The nature of BLB resistance caused by *Xanthomonas* can be seen based on the length of the lesion, the affected area and the disease index (Ogawa *et al.*, 1988). Xoo attack rates on rice plants are strongly influenced by temperature and humidity conditions during infection, the background of plant genetics and the concentration of inoculation and the level of virulence of the disease to plants (Ogawa *et al.*, 1988).

Fig. 1. The length of lesion on F_2 progenies of plant leaves after 3 weeks of Xoo inoculation.

Agronomic Character of F_2 Progenies

Based on data analysis (Table 2) shows that the lowest number of productive tillers is found in T13 and T14 which are 6 tillers. While the highest tillers were found at T22 which reached 12 tillers. The longest panicle was found in T13 and the shortest panicle was found at T15. T23 has the highest grain weight which reaches 55.05 g. The weight of grain per clump is related to the yield potential of rice plants.

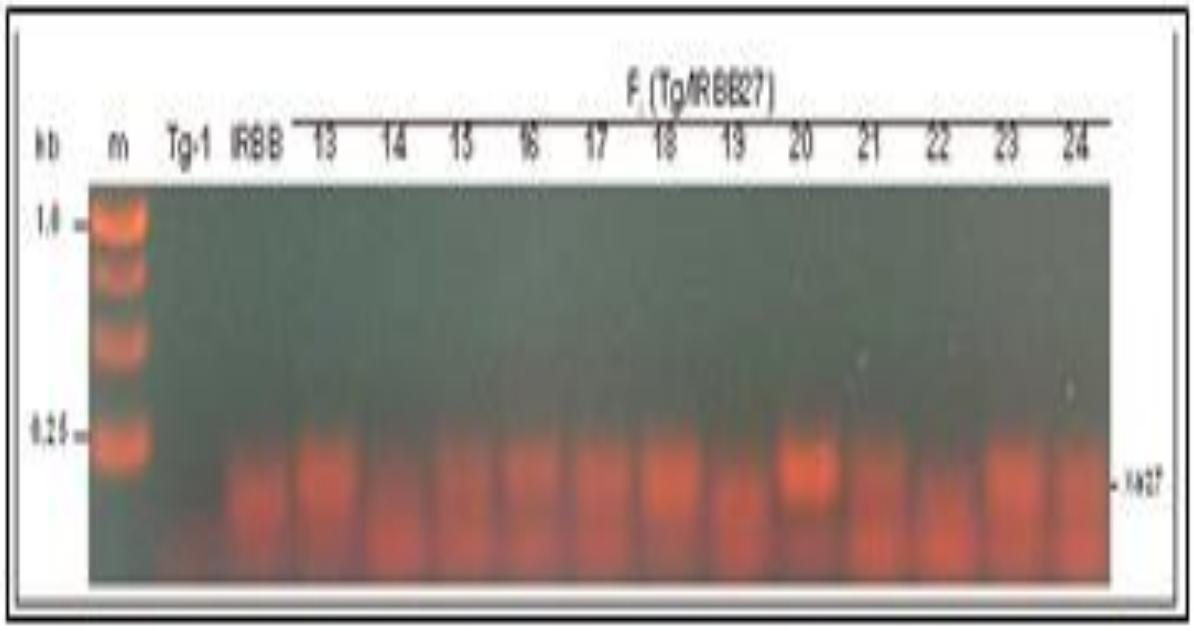

Fig. 2. Molecular analysis the inheritance of *Xa27* gene F_2 progenies crossed between IRBB27 and Ting gong

The higher the weight of grain per clump, the higher the production. While the lowest grain weight was found at T14 which was 17.08 g. T14 is a plant that is susceptible to Xoo stain IIIV from the 12 plants tested. As a result of infection, Xoo strain VIII has caused low weight of grain at T14. T14 also has 1000 grain weight which tends to be low at 22.70 g. Zhao *et al.*, 2007 states that quantitatively, BLB disease causes a low weight of 1000 seeds from affected plants and results in disruption of the cooking process of grain in rice plants (Ou, 1985; Kadir, 2009). Besides that plants that are susceptible to Xoo have a higher percentage of unhulled grain.

This is because Xoo infection in the generative phase will cause disruption of the cooking process. The number of leaves damaged by Xoo attacks causes disruption of photosynthesis in plants so that the yield potential is reduced. High production is the most expected achievement in the assembly of varieties. T23 is the highest production individual of the 12 plants tested. T23 has higher yield potential than Ting gong and IRBB27. The lowest production was found in T14 which only reached 4.27 tons h^{-1}.

Conclusion

T13 and T23 individuals genetically inherit the *Xa27* gene and these two individuals are agronomically resistant to Xoo strain VIII and have high production so that it has the opportunity to be developed into superior varieties.

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