Detection of Xa-27 gene in rice progenies F4 from hybridization of tinggong/ IRBB27 and its resistance to bacterial blight

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Abstract. Tinggong, one of Acehnese rice variety which has long-life span, low production, high stems, and susceptible to bacterial blight (BB). To improve its performance, Tinggong has been crossbred with IRBB27 which has resistance gene (Xa-27) to BB. Five genotypes (T2, T3, T10, T18, T22) of F4 from the cross were used in this research. The existences of Xa-27 gene in the plants were detected molecularly using dominant STS (Sequence-Tagged Site) marker M964e. Phenotypic analysis of plants resistance to BB were performed by inoculation of Xoo strain IV and VIII at 15 DAT and analysed their lesion at three weeks of inoculation. Prospective genotypes were identified based on their agronomic characters. The results show that from 60 plant samples observed, 47 plants (78,33%) from the cross inherited Xa-27 gene, and 34 plants (56,6%) showed resistance to both Xoo strain IV and VIII. The study also revealed that there were 8 plants from this genotypes has potential to be developed. These plants showed resistance to both Xoo strain IV and VIII, harvesting time from 108-116 days after sowing, with estimated production from 6,3-9,4 ton ha⁻¹. These genotypes were T2.10; T3.12; T10.9; T18.1; T18.2; T18.7; T18.10 and T22.3.

1. Introduction
Aceh is the western part of Sumatera island that have a lot of prominent local variety of rice. Local rice variety ‘Tinggong’, is one of popular rice variety from south-western part of Aceh due to its pleasant aroma, having soft and tender texture of cooking. However, this variety has a lot of other character should be developed such as increasing harvest index, reducing plant height and decreasing harvesting time and improving the resistance to BB.

BB is one of the most important diseases which is found in rice plants, this disease is caused by the bacteria Xanthomonas oryzae pv (Xoo). Xoo attacks the young leaves through leaf wounds or natural leaf holes called stomata. This pathogen is spread in the lowlands and highlands, appears in the rainy
season and the dry season and attacks from nurseries to harvesting stage [1]. The attack of pathogens is characterized by the formation of wet lines on the edges of the leaves, then turning whitish yellow. This pathogen caused the diseases in the whole stage of plant development including seedling, vegetative, booting, flowering and ripening stages, by showing the leaves turn pale yellow, wilt, then die.

If the infection occurs when the plants in the flowering stage, the grain is not completely filled as well as caused the increasing of empty grains. The rice plants that were attacked by *Xoo* can cause a low weight of 1000 seeds, reduce the quality of grain and cause high yield losses in rice production. In Indonesia, the attack of BB from 2010 to 2014 reach about 111,738.60 ha [2]. Yield losses in rice production caused by BB diseases vary between 15-80%, depending on the crop development stage in which the disease occurs. The threshold of damage caused by BB in plants is 20% for two weeks before harvest, if this threshold increases every 10%, then the yield loss will increase by 5-10% [3]. The high yield loss is caused by BB disease, the most effective cheap and environmentally friendly method to control this diseases is by using resistant varieties [4].

The use of resistant varieties has not yielded satisfactory results. *Xoo* has a high level of pathotype diversity due to environmental factors. The existence of various environmental factors, several groups strains *Xoo* were created [5]. The dominant strain groups in Indonesia are strain III, strain IV, and Strains VIII. Strains III is the dominant strain in South Sulawesi. Strains IV is a virulent strain in Java, Bali and South Sulawesi. The existence of resistance genes such as Xa1, Xa2, Xa4, Xa7, Xa10, Xa11, Xa14 and Xa27 in rice plants will be able to increase the resistance to the rice plants. Meanwhile, strain VIII is the dominant in West Java. Strains VIII have virulence genes that can break resistance genes Xa1, Xa2, Xa3, Xa4, Xa7, Xa10, Xa11, Xa14 and Xa27 [2]. Gu et al., [6], Jiang et al. [7] stated that the Xa27 resistance gene is a gene found in IRBB27 rice plants which has a wide range of resistance levels to BB. The use of IRBB27 as a source of resistance genes to BB is a very effective way to improve the performance of rice. Therefore, the BB resistance gene (Xa27) can be used for improvement of local rice varieties, such Tinggong.

2. Materials and methods

2.1 Research implementation and genetical resources

The research was conducted in experimental farm as well as in the laboratory of genetics and Plant Breeding, Faculty of Agriculture, Syiah Kuala University from May 2019 to March 2020. The seeds used in this study were 5 genotypes of F4 progenies from cross breeding between Acehnese rice cultivars Tinggong and introduced variety IRBB27. The progenies used in this study were T2, T3, T10, T18, and T22. Tinggong and IRBB27 were also used as comparison for plant analysis.

2.2 Plants cultivation technique

F4 seed were transplanted in soil mix with manure with ratio 2:1 after 18 days of sowing. In one pot contained 5 kg soil and manure were planted 2 seedling with 1 seedling in each planting hole. The basal fertilizer where used NPK and Urea 2.6 g (450 kg ha\(^{-1}\)) and 0.6 g (100 kg ha\(^{-1}\)) respectively one day before transplanting. Additional fertilizers were applied at 14, 28, and 42 days after transplanting (DAT) by using only Urea each 0.6 g pot\(^{-1}\).

2.3 Xoo bacteria inoculation and observation

*Xoo* application was carried out by cutting the tips of young rice leaves at 15 (DAT) about 3-5 cm using sterile scissors dipped in a suspension solution of *Xoo* Strains IV and Strains VIII. The cutting were performed to four leaves in one sample, two leaves for Strains IV analysis and two leaves for Strains VIII analysis. *Xoo* application was performed in the morning to reduce evaporation. *Xoo* observations were carried out by counting the length of the lesions observed at days 21 days after inoculation. The measurement of the length of the lesion were performed from the cut tip of the leaf to the base of the leaf which shows the *Xoo* attack and was expressed in units of cm.
2.4 DNA extraction
The DNA was extracted from the leaves of each 2 weeks-old rice plant sample using a modified protocol as described by Zheng [8]. Taken samples of rice plants about 0.1-0.2 g of young leaves were cut and put inside the 2 ml microcentrifuge tube containing one stainless steel bead. Then the tube containing the leaves of the sample plant were inserted in a microtube box and then stored in the freezer temperature -86 °C for 24 hours. Then the sample of the rice plant is destroyed by shaking the sample box containing the microcentrifuge tube 20-30 times until the rice leaf sample was destroyed. Then add 200-400 μl TPE buffers into each tube containing the crushed sample and the samples were incubated in water bath with a temperature of 65 °C for 20 minutes. After the incubation, then the sample were separated by centrifuge with 13,000 g for 10 minutes. Then the supernatant containing DNA as much as 100 μl was inserted in a 1.5 ml microtube.

2.5 Molecular marker and polymerase chain reaction (PCR) amplification
The molecular marker used in this study was dominant STS (Sequence-Tagged Site) marker M964e for Xa27 gene analysis [9]. Oligo primers for PCR amplification of the molecular markers had DNA sequence Uni F (5'TGT GCA ATG CAG GAT TTC AGT TAC T3') and Uni R (5'TTT CAC CTG CAT AAT GCA AAA GCT AA3'). PCR was performed using EmeraldAmp PCR Master Mix (Takara). For the PCR reactions, the mixtures were initially denatured at 94°C for 120 second followed by 40 cycles of PCR amplification with the following parameters: a 60 s of denaturation at 94°C, a 45 s of annealing at 37 °C, and 90 seconds of primer extension at 72°C. Finally, the reaction mixture was maintained at 72°C for 5 min before completion. The amplified product was electrophoretically resolved on 1 % agarose gel in 0.5×TAE buffer. The Electrophoresis monitor was set up with 100 Volt PAC power for 25-35 minutes. DNA were visualized under UV transilluminator light and documented with a Canon G11 camera.

2.6 Agronomic character analysis
Agronomic performance of F4 progenies for each plant in each genotype were collected from experiment field in dry season (August 2019). Analysis of plant phenotypic character was done on flowering and harvesting time. Others variables analysed including plant height at harvesting time, weight of seed per hill, and estimated potential yield ha⁻¹.

3. Results and discussion
3.1 Analysis the presence of Xa27 gene in F4 progenies of tinggong and IRBB27
Molecular analysis the presence of Xa27 gene in F4 progenies of Tinggong/IRBB27 for each plant of genotype T2, T3, T10, T18 and T22 is shown in Fig. 1

Fig. 1 showed that, among 60 analysed sample in genotype (T2, T3, T10, T18 and T22), 47 samples showed the presence of Xa-27 gene in the progenies. In F4 Tg/IRBB27 genotype T2, among 12 plants to be analysed there were 8 plants (66%) genetically inherited Xa27 gene from IRBB27 male parent #3, 4, 5, 6, 7, 10, 11 and 12. In genotype T3, there were 4 plants inherited Xa27 genes (33.3%) #5, 10, 11 and 12. Moreover, in genotype T10, there were 7 plants (58.3%) inherited the Xa27 gene #1, 2, 3, 5, 6, 8, and 9. Whereas in genotype T18 there were 6 plants (50%) inherited Xa27 genes #1, 8, 9, 10, 11 and 12 and in genotype T22 all of 12 plants (100%) inherit the Xa27 gene from its male parent #1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12.
Figure 1. Genetic analysis of Xa27 gene from Tinggong, IRBB27, and F₄ Progenis Tg/IRBB27

Lesion analysis and agronomic character of F₄ progenies Tg/IRBB27 for 5 genotypes (T₂, T₃, T₁₀, T₁₈ and T₂₂), are shown in table 1. The table showed that in genotype T₂ there were 5 plants # 2, 3, 4, 10 and 12 have length of lesion for Xoo strains IV ranging from 0.25-1.55 cm or more resistance than their parents Tinggong or IRBB27. In addition, among 12 plants analysed for resistance to Xoo strain VIII, it showed that there were 10 plants (83.3%) # 1, 2, 3, 4, 7, 8, 9, 10, 11 and 12 had lesion lengths ranging from 0.30-2.70 cm, or more resistant than their parent Tinggong and IRBB27.

Table 1. Lesion analysis and agronomic character of F₄ progenies Tg/IRBB27 for 5 plant genotype.

| Genotypes       | Lesion length (cm) and disease phenotype | Heading date (DAS) | Harvesting time (DAS) | Plant height (cm) | Weight of filled grains per plant (g) | Estimated yield (t/ha) |
|-----------------|------------------------------------------|-------------------|-----------------------|------------------|---------------------------------------|------------------------|
|                 | Xoo Strain IV                            | Xoo Strain VIII   |                       |                  |                                       |                        |
| Tinggong        | 1.39 ± 0.40 R                            | 3.28 ± 1.10 MR    | 132.67                | 162.67           | 157.50                                | 26.19                  | 6.55                   |
| IRBB27          | 1.47 ± 0.94 R                            | 1.23 ± 0.47 R     | 75.33                 | 105.33           | 84.08                                 | 19.45                  | 4.86                   |
| F₄ Tg/IRBB27    | 11.55 ± 4.45 S                           | 0.65 ± 0.35 R     | 86                    | 116              | 108.0                                 | 16.81                  | 4.20                   |
| T₂              | 1.70 ± 0.10 R                            | 0.25 ± 0.05 R     | 76                    | 106              | 85.0                                  | 12.14                  | 3.04                   |
| 3               | 0.65 ± 0.15 R                            | 0.40 ± 0.10 R     | 75                    | 105              | 92.0                                  | 12.41                  | 3.10                   |
| 4               | 0.40 ± 0.10 R                            | 0.45 ± 0.15 R     | 91                    | 121              | 91.5                                  | 17.44                  | 4.36                   |
| 5               | 0.45 ± 0.15 R                            | 7.90 ± 7.60 MS    | 77                    | 107              | 90.0                                  | 7.80                   | 1.95                   |
| 6               | 1.15 ± 0.65 R                            | 5.10 ± 3.40 MR    | 78                    | 108              | 85.0                                  | 14.18                  | 3.55                   |
| 7               | 9.40 ± 7.10 S                            | 1.75 ± 1.55 R     | 81                    | 111              | 104.0                                 | 20.56                  | 5.14                   |
| 8               | 6.30 ± 4.70 MS                           | 0.30 ± 0.00 R     | 83                    | 113              | 94.0                                  | 20.31                  | 5.08                   |
| 9               | 4.75 ± 3.35 MR                           | 0.85 ± 0.15 R     | 84                    | 114              | 94.5                                  | 13.98                  | 3.50                   |
| 10              | 0.25 ± 0.15 R                            | 0.60 ± 0.10 R     | 84                    | 114              | 116.0                                 | 32.14                  | 8.04                   |
|    | 11  | 12   |
|----|-----|------|
| F<sub>t</sub> | 3.50 ± 2.70 R | 0.75 ± 0.15 R |
| Tg/IRBB<sup>27</sup> | 0.90 ± 0.60 R | 0.95 ± 0.25 R |
| T<sub>3</sub> | 0.90 ± 0.40 R | 0.50 ± 0.10 R |
| 4 | 0.45 ± 0.05 R | 5.25 ± 3.05 MR |
| 5 | 1.40 ± 1.00 R | 0.35 ± 0.15 R |
| 6 | 7.05 ± 0.95 MS | 0.45 ± 0.25 R |
| 7 | 1.95 ± 1.55 R | 0.40 ± 0.10 R |
| 8 | 0.30 ± 0.00 R | 1.70 ± 1.30 R |
| 9 | 7.00 ± 6.70 MS | 0.35 ± 0.05 R |
| 10 | 0.80 ± 0.30 R | 0.35 ± 0.05 R |
| 11 | 4.50 ± 2.00 MR | 2.10 ± 0.90 R |
| 12 | 1.00 ± 0.70 R | 2.30 ± 1.70 R |

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| F<sub>s</sub> | 6.30 ± 4.70 MS | 10.75 ± 10.25 S | 18.75 ± 3.75 S | 26.75 ± 1.05 S | 22.65 ± 2.45 S | 1.00 ± 0.50 R | 3.40 ± 2.10 MR | 2.60 ± 2.40 R | 1.80 ± 0.90 R | 0.70 ± 0.00 R | 6.60 ± 4.60 MS | 1.60 ± 1.00 R |
| Tg/IRBB<sup>27</sup> | 6.30 ± 4.70 MS | 10.75 ± 10.25 S | 18.75 ± 3.75 S | 26.75 ± 1.05 S | 22.65 ± 2.45 S | 1.00 ± 0.50 R | 3.40 ± 2.10 MR | 2.60 ± 2.40 R | 1.80 ± 0.90 R | 0.70 ± 0.00 R | 6.60 ± 4.60 MS | 1.60 ± 1.00 R |
| T<sub>10</sub> | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R |
| T<sub>18</sub> | 0.40 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R | 0.70 ± 0.40 R |

|    | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  |
|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| F<sub>d</sub> | 1.05 ± 1.60 R | 2.10 ± 1.60 R | 0.70 ± 1.45 R | 0.90 ± 0.05 R | 1.70 ± 1.90 R | 1.80 ± 0.15 R | 0.70 ± 0.25 R | 0.95 ± 1.35 R | 0.70 ± 0.05 R | 0.35 ± 0.15 R | 0.60 ± 0.90 R | 1.15 ± 0.55 R |
| Tg/IRBB<sup>27</sup> | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R | 2.10 ± 1.60 R |
| T<sub>22</sub> | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R | 0.40 ± 0.10 R |

*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) [10].
Lesion length analysis also shown that, in genotype T3, there were eight plants (66.6%) showed their resistance to Xoo strain IV with lesion length ranging from 0.30 – 1.95 cm # 2, 3, 4, 5, 7, 8, 10 and 12 and there were 11 plants (96.6%) # 1, 2, 3, 5, 6, 7, 8, 9, 10, 11 and 12 with lesion lengths ranging from 0.35-2.30 cm by inoculation with Xoo strain VIII. Whereas, in genotype T10, there were six plants (50%) # 5, 7, 8, 9, 11 and 12 showed resistance to Xoo strain IV with lesion lengths ranging from 0.30-2.60 cm, and also there were six plants (50%) # 2, 4, 6, 9, 10 and 12 had lesion lengths ranging from 0.35-2.75 cm, almost similar to their male parent IRBB27. In genotype T18 however, among 12 plants observed to analysed the resistance to Xoo strain IV and strains VIII, all plants (100%) show resistance to Xoo with lesion length ranging from 0.35-2.60 cm. In genotype T22, showed that all plants (100%) had the resistance to Xoo strains IV, with lesion lengths ranging from 0.25-2.15 cm. Based on resistance analysed to Xoo strains VIII, it showed that there were nine plants (75%) # 1, 3, 4, 6, 8, 9, 10, 11 and 12 had lesion lengths ranging from 0.40 - 2.40 cm almost similar to their parents IRBB27. This suggests, of the two strains used, plants are more resistant to Xoo strains IV than strains VIII. Yuliani and Natasuwiry [11] conclude that the resistance reactions of varieties are grouped based on the severity of the disease in the last observation.

From Table 1, also showed that from 12 plants of genotype T2 there is one plant that has the potential to be developed further (#10) because this plant, had lifespan of 114 days after sowing (DAS), a short plant height of 116 HSS and has a high production per ha of > 8.04-ton ha⁻¹. Whereas in genotype T3 there is one plant that has the potential to be developed (Plant no 12) because it has a lifespan of 108 DAS, a short plant height oand has estimated yield per ha of 6.28 tons/ha.

In genotype T10 however, among 12 plants, there were seven plant (58.3%), # 1, 2, 6, 7, 8, 9 and 10 which the life span ranges from 110 – 119 DAS and there were five plants (41.6%) with their life span from 134-136 DAS (# 3, 4, 5, 11 and 12) or almost similar to their male parent IRBB27. Among the plants in genotype T10, there were 7 plants (58.3%), # 3, 4, 6, 7, 8, 9 and 12 have moderate height ranging from 111-130 cm almost similar to IRBB27. Four plants (33.3%) # 1, 2, 10 and 11 have a height category ranging from 132 - 141 cm, similar to female parents Tinggong with height were 157.50 cm. Then, In genotype T10, there were four plants (33.3%) had estimated potential yield reach 7.35–7.90 ton ha⁻¹. These plant were plant number # 6, 7, 9 and 10. Based on the criteria of yield per hectare, these four plants are included in the high criteria, one plant enters the moderate criteria and seven plants enter low rite-ria.

Moreover, in genotype T18 among 12 plant, all of the plant had the life span between 82-121 DAS. These plants were number # 1, 2, 3, 4, 5, 6, 7, 8, 10, 11 and 12. In this genotype there were six plants (49.9%) has short plants ranging from 76 - 108 cm or almost similar to their parent IRBB27. These plants were number # 1, 4, 6, 10 and 11 and 12. While six plants (50%) i.e. plants number 2, 3, 5, 7, 8 and 9 have a medium plant height ranging from 111-119 cm, exceeding it male parent IRBB27. In genotype T18, there is one plant (8.3%) 6.55 tons ha⁻¹ is found in plant number 1 with a higher yield of 9.36 tons ha⁻¹, three plants (25%) # plants number 2, 7 and 10 wi yields ranging from 6.37-6.54 tons ha⁻¹.

In genotype T22, all of the plants (100%) had the life span between 111-120 DAS. In this genotype, there were eleven plant (91.6%) had the plant height 102 - 129 cm, and only one plant (8.3%) (Plant number # 5 that had plant height 131 cm. Moreover, in genotype T22, there was one plant # plant number 3 with estimated yield reached 8.33 tons ha⁻¹, five plants (41.6%) # 7, 9, 10, 11 and 12 had the yield ranging from 4.62-5.93 tons ha⁻¹. Then six plants (50%) had the yield less than 3.5 ton ha⁻¹. Based on the criteria of yield per hectare, this plant is included as medium and low criteria of yields.

4. Conclusions
Based on molecular analysis it shows that 78.3% samples of F₄ Tg / IRBB27 inherited the Xa27 gene. Whereas based on lesion length analysis its showed that 75% samples of F₄ Tg / IRBB27 resistance to Xoo strain IV and 85% resistance to Xoo strain VIII and 56.6% plants showed resistance both to Xoo strain IV and VIII. There were 7 plants from 5 genotypes are prospective to be developed further because
of these plants resistance to both Xoo strain IV and VIII, estimated production from 6.3-9.4 ton ha\(^{-1}\), harvesting time from 108-116 days after sowing (DAS) with plant height about 104-118 cm.

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