Identification of Blast Resistance Genes in Fifteen Rice Accessions (*Oryza sativa* L.) from North Sumatera

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Abstract. One challenge in improving rice productivity is the occurrence of blast disease caused by *Pyricularia grisea*. Development of current disease is affected by several factors, one of which is an expression of resistance gene towards blast by rice systemic defense. In order to construct blast-resistant variety, research has been conducted to identify resistance-controlling gene against blast in fifteen rice accessions native to North Sumatera. Identification of blast resistance gene performed using PCR amplification of rice genome with six specific primers: *Pi*-d2, *Pup1*, *Pi*-ta2, *Pi*-37, *Pi*-z, and *Pib*. The results showed that genes, *Pi*-d2, *Pi*-z, and *Pib* were present on fifteen rice accessions, gene *Pup1* positively located in fourteen accessions (93%), *Pi*-ta2 in thirteen accessions (87%), and *Pi*-37 in seven accessions (47%). Rice accessions with six blast resistance genes detected were: *Ampari* 3, *Bagendi* 1, *Bagendi* 3, *Martabe* 3, *Siporang* 2, and *Silatian* 2. The least number of genes were detected from two accessions namely: *Ampari* 2 and *Sirara* 2, with only four resistance genes.

1. Introduction

Rice is a staple plant for Indonesians. North Sumatra, as one of rice cultivation center in Indonesia, holds a number of rice collections commonly planted by rice farmers. The existence of rice is important as a germplasm source for assembling superior rice cultivars to support the food self-sufficiency in Indonesia. Recently, the existence of rice from North Sumatra is still less informative, along with the limited study upon finding superior cultivars as a source of valuable germplasms [1].

One of the desirable functional traits in rice to achieve food self-sufficiency is disease resistance. Blast disease caused by fungus *Pyricularia oryzae* Cav. [synonym *Magnaporthe oryzae* (Hebert) Barr] is one of the important diseases in rice plants throughout the world [2]. Blast fungus can infect rice plants at each growing stage by forming patches on leaves, buds, panicle rots, foliar rots and grains that caused empty viable seeds leading to crop failure. In Indonesia, blast disease has spread in almost all rice production centers [3].

Naturally, certain rice varieties may show a considerable resistancy towards blast disease. Previous study revealed that a gene identified as *Pup1* locus (P uptake 1) in the local varieties of rice, namely Kasalath variety in which located on chromosome 12 [4]. Based on sequence analysis, it is also known that at the *Pup1* locus there are dirigent-like protein genes which may play an important role in enhancing the plant's resistance mechanism to abiotic (drought, aluminum) and biotic stresses (blast disease, bacterial leaf blight, and cork-stem borer) [5]. Other study also reported that resistance to blast
disease in rice cultivars under expression of Pup1 locus was clearly seen in hybrid of Situ Bagendit variety [6].

Further analysis of genes controlling blast disease resistance in wild rice species Oryza rufipogon, was also conducted for mapping the QTL specific for blast in the BC2F3 population of the rice variety, IR64 [7,8]. Eleven blast resistance genes had been screened on 32 rice accessions from China, namely Pi-d2, Pi-z, Pi2-t, Pi-9, Pi-36, Pi-37, Pi5, Pi -b, Pik-p, Pik-h and Pi-ta [9]. Currently, there is still less information on the presence of blast disease resistance genes in local rice of North Sumatra. The finding of potential or superior rice through molecular detection of blast resistance genes may result in the use of germplasm sources to assemble future superior rice in Indonesia.

2. Materials and Methods

2.1. Rice materials and DNA genom isolation

Rice collections used in this study are presented in Table 1. The samples were soaked for 24 hours, and planted in plastic containers filled with a mixture of 50% soil + 50% compost. Each containers were covered with dark plastic. The covers were opened three days after planting. Standard nursing and water administration were given daily in the morning. DNA samples were obtained by sampling several foliar specimens 10 days after planting.

Table 1. Information of rice collections used in this study

| N | Accessions | Code | Origins (District, Regency) |
|---|------------|------|-----------------------------|
| 1 | Ampari 1   | A1   | Batang Angkola, Tapanuli Selatan |
| 2 | Ampari 2   | A2   | Batang Angkola, Tapanuli Selatan |
| 3 | Ampari 3   | A3   | Sipirok, Tapanuli Selatan |
| 4 | Bondo      | B    | Batang Angkola, Tapanuli Selatan |
| 5 | Sibidas    | D1   | Sigalangan, Tapanuli Selatan |
| 6 | Bagendi 1  | G1   | Batang Angkola, Tapanuli Selatan |
| 7 | Bagendi 2  | G2   | Padangsidimpuan, Tapanuli Selatan |
| 8 | Bagendi 3  | G3   | Batang Angkola, Tapanuli Selatan |
| 9 | Martabe 1  | M1   | Batang Angkola, Tapanuli Selatan |
| 10 | Martabe 3  | M3   | Binanga, Padang Lawas |
| 11 | Siporang 2 | P2   | Sipirok, Tapanuli Selatan |
| 12 | Sirara 1   | R1   | Hutaimbaru Tapanuli Selatan |
| 13 | Sirara 2   | R2   | Binanga Padang Lawas |
| 14 | Sayuti     | S    | Padangsidimpuan Batunadua, Tapanuli Selatan |
| 15 | Silatian 2 | L2   | Portibi Padang Lawas Utara |

DNA isolation was performed using the CTAB (Cetyl Methyl Ammonium Bromide) method according to the modified protocol [10]. A sample weighing 0.1 g of rice foliars were cut into small pieces and put into a mortar containing 700 µL 2% CTAB extraction buffer (20 mM EDTA, 0.1 M Tris-HCL pH 8.0, 1.4 M NaCl, 2% CTAB, and 0.4% β-mercaptoethanol) pre-heated at 65°C, then crushed using pestle. The sample was then placed into 1.5 mL microtube and incubated at 65°C for 45 min following a vortex in each 15 min, then added with 500 µL phenol-chloroform-isoamyl alcohol (25:24:1) and vortexed. Samples were centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was transferred to a new tube and added again with 500 µL of phenol-chloroform-isoamyl alcohol (25:24:1). At this stage, procedure was repeated two times. Then the supernatant was transferred to a new tube and added with 700 µL of cold isopropanol and incubated in the freezer for 2 hr. Samples were centrifuged at 12,000 rpm for 10 min at 4°C. The pellets were washed using 70% alcohol and centrifuged again at 12,000 rpm for 10 min at 4°C. The pellets were dried and dissolved with 100 µL aqua-bidest and incubated for 1 hr at 37°C and stored at -20°C as stock.
The qualitative and quantitative tests of DNA genomes were evaluated by agarose gel electrophoresis (1%) and nanophotometer (IMPLEN P-360 Nano-Photometer P-Class) with a wavelength of 260 nm. Then the DNA is calculated for purity with an absorbance ratio of 260/280 ($A_{260}/A_{280}$). The electrophoresis gel visualization was performed under the UV transiluminator and results were documented.

2.2. DNA amplification and analysis

PCR-DNA amplification used the Master mix PCR kit (Promega, US) with a final volume of 25 µL. The PCR program was used as follows: 35x cycles of amplification with initial denaturation at 94°C for 1 min, annealing at 51.3–55.5°C for 45 sec, elongation at 72°C for 1 min and final elongation at 72°C for 5 min. Primers used in this study were 6 primary pairs, namely Pi-d2, Pup1, Pi-ta2, Pi-37, Pi-z, and Pib [9,11–14]. Specifications of each primers are presented in Table 2.

Table 2. Characteristics of PCR primers for detecting blast resistance genes

| No. | Primers | Sequence (Forward/Reverse) | Annealing temperature (°C) |
|-----|---------|---------------------------|---------------------------|
| 1.  | Pi-d2   | F: TTGGCCTATCATAGGCGTCC   | 57°C                      |
|     |         | R: ATTTGAAGGCGTTTGCGTAGA  |                           |
| 2.  | Pup1    | F: TCTAAAAATTTCCTCAGTGATGTACTCC | 59°C                  |
|     |         | R: TTOGGGTGATCAGCTTTCAGA  |                           |
| 3.  | Pi-ta2  | F: AGCAGGTTATAAGCTAGGCC  | 59°C                      |
|     |         | R: CTACCAACAAGTTGTCATCAA  |                           |
| 4.  | Pi-z    | F: GGACCCCGCTTTCCACGTGTAAC | 63°C                   |
|     |         | R: AGGAATCTATTGCTAAGCATGAC |                       |
| 5.  | Pi-37   | F: TCTTGAGGGTCCCAGTGTAC  | 61°C                      |
|     |         | R: CGAACAGTGGCTGGTATCTC  |                           |
| 6.  | Pib     | F: GACTCGGTCGACCAATTCGCC | 65°C                      |
|     |         | R: ATCAGGCCAGGCCAGATTTTG |                           |

PCR amplification results were separated in 1.5% agarose gel in TAE buffer (Tris-Acetate EDTA) 1x with a voltage of 70 V for 60 min. The gel is soaked in EtBr for 10 min. The gel is soaked again with aquabides for 5 min in order to clarify the color of DNA. The gel is visualized under the UV transiluminator and documented.

3. Results and Discussions

3.1. DNA isolation of local rice collections in North Sumatera

Fifteen collections of local rice from North Sumatra were isolated by CTAB method, and were analyzed for its DNA quantity and quality. The results of DNA isolation showed a thick and an intact band in the upper position or just below the gel electrophoresis well (Figure 1.).

Figure 1. DNA genome electrophoresis results: Ampari 1 (A1), Ampari 2 (A2), Ampari 3 (A3), Bondo (B), Sibidas (D1), Bagendi 1 (G1), Bagendi 2 (G2), Bagendi 3 (G3), Martabe 1 (M1), Martabe 3 (M3), Siporang 2 (P2), Sirara 1 (R1), Sirara 2 (R2), Sayuti (S), Silatian 2 (L2)

The integrity of DNA bands obtained from the visualization of electrophoresis results showed the success of DNA genome isolation. The whole band is ready to be used for further analysis. The presence of single band showed that the isolated genomic DNA can be used as a PCR template to
analyze the presence of blast resistance gene fragments. The quantity and purity of the DNA genomes is presented in Table 3.

Table 3. Quantity and purity of total DNA of rice collections in North Sumatera

| N  | Accessions | Codes | Concentration | Purity |
|----|------------|-------|---------------|--------|
| 1. | Ampari 1   | A1    | 9,600 ng/µL   | 2,105  |
| 2. | Ampari 2   | A2    | 15,8 ng/µL    | 2,036  |
| 3. | Ampari 3   | A3    | 5,570 ng/µL   | 2,025  |
| 4. | Bondo      | B     | 19,1 ng/µL    | 1,963  |
| 5. | Sibidas    | D1    | 3,900 ng/µL   | 2,131  |
| 6. | Bagendi 1  | G1    | 5,470 ng/µL   | 2,064  |
| 7. | Bagendi 2  | G2    | 5,980 ng/µL   | 2,055  |
| 8. | Bagendi 3  | G3    | 2,830 ng/µL   | 2,081  |
| 9. | Martabe 1  | M1    | 58,4 ng/µL    | 2,069  |
| 10.| Martabe 3  | M3    | 19,3 ng/µL    | 1,967  |
| 11.| Siporang 2 | P2    | 5,290 ng/µL   | 2,058  |
| 12.| Sirara 1   | R1    | 5,790 ng/µL   | 2,105  |
| 13.| Sirara 2   | R2    | 15,3 ng/µL    | 2,004  |
| 14.| Sayuti     | S     | 13,3 ng/µL    | 2,070  |
| 15.| Silatian 2 | L2    | 21,1 ng/µL    | 1,890  |

Based on the results, it can be seen that through DNA genom isolation procedure yielded a purity of 1.890–2.105, and a concentration of 13.3–9,600 ng/µL. The results indicated that DNA genomes are free of contamination and produce sufficient quantity for PCR analysis.

3.2. Detection of blast resistance genes from local rice collections of North Sumatera

Detection of the presence of blast resistance genes was carried out on 15 rice collections from North Sumatra using six specific primers of blast disease resistance genes in rice. The results show that the six primers can detect the presence of genes Pi-d2, Pup1, Pi-ta2, Pi-37, Pi-z, dan Pib (Figure 2).

Figure 2. PCR amplicons of blast-resistance genes using: A Primer Pi-d2, B. Primer Pup1, C. Primer Pi-ta2, D. Primer Pi-37, E. Primer Pi-z, and F. Primer Pib. (M=Marker 100bp, A1= Ampari 1, A2= Ampari 2, A3= Ampari 3, B= Bondo, D1= Sibidas 1, G1= Bagendi 1, G2= Bagendi 2, G3= Bagendi 3, M1= Martabe 1, M3= Martabe 3, P2= Siporang 2, S= Sayuti, L2= Silatian 2).

The positive gene genes Pi-d2, Pi-z, and Pib were all (15) the North Sumatra rice collection analyzed, 14 positive Pup I genes (93%), positive Pi-ta at 13 rice collections (87%), and the Pi-37 gene was positive in 7 rice collections (47%). Yan et al. (2017) have reported that the 100% positive Pi-d2
and Pi-\(z\) gene in 32 Chinese rice collections which have blast resistance, and Pi-37 included a low presence which is only positive in 4 rice collections of 32 rice accessions analyzed. There were 6 positive detected rice collections containing the six genes detected, namely Ampari 3, Bagendi 1, Bagendi 3, Martabe 3, Siporang 2, and Silatian 2 collections (Table 4). While Ampari 1 rice is not positive for the Pup-1 gene; Ampari 2 and Sirara 2 are not positive Pi-ta2; Ampari 2, Bondo, Sibidas, Bagendi 2, Martabe 1, Siara 1, Sirara 2, and Sayuti were not positive for the Pi-37 gene.

Table 4. Detection of blast resistance genes of rice collections in North Sumatera

| No. | Accessions | Pi-\(d2\) | Pup1 | Pi-ta2 | Pi-37 | Pi-\(z\) | Pib |
|-----|------------|----------|------|--------|-------|---------|-----|
| 1.  | Ampari 1   | \(+\)     | \(-\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 2.  | Ampari 2   | \(+\)     | \(-\) | \(-\)   | \(+\)  | \(+\)   | \(+\) |
| 3.  | Ampari 3   | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 4.  | Bondo      | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 5.  | Sibidas    | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 6.  | Bagendi 1  | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 7.  | Bagendi 2  | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 8.  | Bagendi 3  | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 9.  | Martabe 1  | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 10. | Martabe 3  | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 11. | Siporang 2 | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 12. | Sirara 1   | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 13. | Sirara 2   | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 14. | Sayuti     | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |
| 15. | Silatian 2 | \(+\)     | \(+\) | \(+\)   | \(+\)  | \(+\)   | \(+\) |

These results indicate that the genes Pi-\(d2\), Pi-\(z\), and Pib are positive in 15 North Sumatra rice collections, positive Pup1 gene in 14 collections and negative 1 collection, Pi-ta2 gene is not positive in 2 collections, and Pi-gene is 37 were positive in 7 collections and negative in 8 other collections. This shows the diversity of the presence of resistance genes against blasts in rice collections from North Sumatra.

It is also showed that there are several collections of rice that have the same name, but in collections from different regions, there is a diversity of the presence of resistance genes to different blasts. Ampari 3 rice collection from Sipirok positively carried the Pi-ta2 and Pi-37 genes, but Ampari 2 from Batang Angkola was negative for both genes. Martabe 3 from Binanga is positive for the Pi-37 gene, but Martabe 1 from Batang Angkola does not contain this gene, neither does Sirara 1 from Hutaimbaru South Tapanuli positive for Pi-ta2 gene, while Sirara 2 from Binanga Padang Lawas is negative Pi-ta2. This is possible because of the mixing of seeds used by farmers. This possibility is reinforced by the reality in the field where adjacent farmers grow different varieties of rice, while rice has open pollination. So pollination can occur between different paddy fields, while farmers prepare rice seeds for further planting from their own crops. This condition allows the flow of genes that produce genetic diversity.

4. Conclusions

All resistance genes to blast in rice detected (6 genes), showed the diversity of the presence of resistance genes against blasts in fifteen rice collections from North Sumatra. The Pi-\(d2\), Pi-\(z\), and Pib genes are positive in 15 rice collections, the Pup 1 gene is positive in 14 collections (93%), positive Pi-ta in 13 rice collections (87%), and Pi-37 gene positive for 7 rice collections (47%). The positive collection of rice to the 6 genes detected was Ampari 3, Bagendi 1, Bagendi 3, Martabe 3, Siporang 2, and Silatian 2. The lowest rice collection detected by the lowest blast disease control gene was rice Ampari 2 and Sirara 2, namely only positive 4 genes.
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