
Exploration of Bengkulu local rice varieties as new germplasm for breeding: insights from agro-morphological traits to blast resistance genes

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Herawati, R., Sumardi., Masdar., Endrawan, R. T., Oktarena, R., Kesuma, S. P., Ganefianti, D. W., Marlin., Romeida, A., Rustikawati. and Herison, C. (2026). Exploration of Bengkulu local rice varieties as new germplasm for breeding: insights from agro-morphological traits to blast resistance genes. International Journal of Agricultural Technology 22(1):161-180.

Abstract The local rice (landrace) germplasm plays a crucial role in the development of superior rice varieties. The unique advantages of local varieties, such as resistance to environmental stress, are valuable assets that must prevent extinction. Therefore, maintaining a collection of local rice germplasm is essential as a genetic resource for breeding new varieties with high yield potential, resistance to pests and diseases, early maturity, and other desirable traits. The exploration identified 30 local rice varieties with diverse agronomic characteristics. Gene identification revealed that all varieties carried the blast resistance genes Pup1 and Pib, while only a few possessed the genes Pita2, Pii, and Pik. Notably, some varieties exhibited multigenic resistance, including Segumai and Tumbar, which were found to be strong potential as new germplasm sources for blast-resistant rice breeding programs.

Keywords: Local rice varieties, Blast disease, Germplasm, Multigenic resistance

Introduction

Bengkulu Province has vast rice fields spread across nine regencies and municipalities, serving as key development areas for both lowland and upland rice cultivation. Previous monitoring has shown that blast infestation is widespread in rice, although its transmission has not been well documented. *Pyricularia grisea*, the pathogenic fungus responsible for rice blast, exhibits significant genetic diversity and is highly adaptive to its host plants (Xiao *et al.*, 2016; Orasen *et al.*, 2020). The development of durable and broad-spectrum blast-resistant rice varieties can overcome this problem. One effective strategy is to create pyramiding genes, i.e., the introgression of several resistance genes in genotypes that can survive various blast races in the field (Xiao *et al.*, 2016; Orasen *et al.*, 2020; Mutiga *et al.*, 2021). The highly dynamic and multi-race nature of the blast pathogen can be managed sustainably by breeding rice varieties with long-lasting, polygenic resistance (Xiao *et al.*, 2015; Orasen *et al.*, 2020; Wang *et al.*, 2023).

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Selecting elders as a source of resistance genes, crossing two or more elders, and assessing offspring are all steps in the breeding process used to create superior rice varieties. However, because of the shift to traditional farming methods and the disregard for local genetic resources (landraces), rice's genetic resources are becoming more and more limited. (Setyowati *et al.*, 2018). It is anticipated that landrace varieties with particular advantageous traits will boost rice productivity in particular regions (Hairmansis *et al.*, 2015). According to Bakhtiar *et al.* (2011), conserving genetic resources is essential for adjusting to changing environmental conditions, satisfying changing consumer needs, and preserving a genetic reservoir for upcoming plant breeding. The availability of plant genetic resources is seriously threatened by global climate change.

In order to create superior rice varieties, the local landrace rice germplasm is essential. A wider gene pool is made possible by high genetic diversity, which makes it possible to create varieties that cater to particular user requirements (Rembang *et al.*, 2018). Farmers still cultivate a number of local rice varieties that have been widely used in rice production (Khairullah *et al.*, 2021). The ability of local varieties to withstand environmental stress is one of their main advantages, making them important genetic resources that need to be protected from extinction (Ahimsya and Basunanda, 2018). A large collection of germplasm raises the possibility of superior varieties being developed (Rembang *et al.*, 2018). Genetic erosion results from the displacement of traditional local varieties due to the breeding of new high-yielding varieties (Nurhasanah, 2015). Because modern varieties frequently lack the distinctive genes and gene complexes found in landraces, genetic erosion takes place. Because local rice germplasm's genetic diversity is a key resource for creating new varieties with desired qualities like high yield, resistance to pests and diseases, early maturity, and other agronomic advantages, it is imperative that it be conserved (Nickolas *et al.*, 2018; Yadav *et al.*, 2019). Systematic research and conservation initiatives are the first step in protecting regional rice varieties.

Several blast resistance genes have been identified, and monogenic lines carrying these genes have been developed as differential varieties (Stam *et al.*, 2014; Wiesner-Hanks and Nelson, 2016; Herawati *et al.*, 2022; Herawati *et al.*, 2024). When a plant has a major R gene, *Pyricularia oryzae* races with the corresponding avirulence (Avr) gene cannot infect it. However, because the pathogen can develop new virulent races through gene recombination or Avr gene mutation, R-gene-mediated resistance is frequently transient (Wang and Valent, 2017). Single-resistance gene varieties offer protection against particular virulent races. Conversely, long-term, broad-spectrum resistance can be conferred by the presence of multiple major resistance genes (Xiao *et al.*, 2016; Li *et al.*, 2020).

Numerous molecular markers have been used to screen for blast resistance genes in rice, and the results showed a strong correlation with 11 known blast resistance genes (*Pi-d2*, *Pi-z*, *Pi-z-t*, *Pi-9*, *Pi-36*, *Pi-37*, *Pi5*, *Pi-b*, *Pik-p*, *Pik-h*, and *Pi-ta2*) (Su *et al.*, 2015; Yan *et al.*, 2017; Jiang *et al.*, 2019; Meng *et al.*, 2020). These genes are essential for studying *P. oryzae*'s genetic diversity and are frequently used as DNA markers, especially sequence-characterized amplified regions (SCAR) (Li *et al.*, 2020;

Kurrata *et al.*, 2021). Multiple blast resistance genes that have the potential to produce durable resistance can be found using molecular markers. This method makes it easier to find pyramiding genes, which can successfully combat a variety of extremely dynamic blast pathogen populations (Xiao *et al.*, 2015; Orasen *et al.*, 2020; Wang *et al.*, 2023). In order to provide new sources of germplasm for upcoming breeding initiatives, this study sought to identify blast resistance genes in Bengkulu local rice varieties (landrace).

Materials and methods

Exploration of Bengkulu local rice varieties

Four Bengkulu Province regencies—South Bengkulu Regency, Central Bengkulu Regency, Rejang Lebong Regency, and North Bengkulu Regency—were the sites of the investigation into regional rice varieties. The relevant agencies (Distan Kabupaten, Bapeluh, community leaders, and NGOs) collected the data, which included primary data (like commonly grown rice varieties) and secondary data (like soil types and climate data) gathered through questionnaires and in-person interviews with conservation farmers and community leaders. In Bengkulu Province, we also conducted inventories of local rice varieties in a few chosen villages, subdistricts, and districts.

The research population consisted of farmers and farmer groups cultivating local rice varieties in Bengkulu Province. The selected local varieties were those commonly cultivated by farmers over the past five years, exhibited favorable agronomic characteristics, were well-liked by farmers, and were native to the region. The collected seed samples were stored for further experimental analysis.

Agro-morphological characteristics

The experiment was conducted to determine the agromorphological characteristics of the local varieties obtained from the exploration. The materials used included 30 local varieties, topsoil as the growing medium, manure, N, P, and K fertilizers, and 10 kg pots. The base fertilizer was applied according to the recommended dosage. Seeds were planted at a rate of one plant per pot, with each variety being planted 10 times. The observed agronomic characteristics included plant height, total number of tillers, number of productive tillers, panicle length, flag length, day flowering, day maturity, total grain per panicle, number of fill grains per panicle, number of empty grains per panicle, weight of 1000 grains, and grain weight per hill. The data were tabulated and averaged, and the standard deviation was calculated using Microsoft Excel. A dendrogram was created to determine the genetic relationships among the varieties, and Pearson correlation analysis was performed using XLSTAT software to examine the relationships between the characteristics.

Identification of blast resistance gene

Five specific primer pairs were applied to detect blast resistance genes: *Pup1*, *Pita-2*, *Pii*, *Pib*, and *Pik* (Table 1). The obtained local rice varieties, as well as the resistant check variety (Situ Patenggang) and the sensitive check variety (Kencana Bali), were planted in plastic tubs. Fresh leaf samples were collected at the age of 2 weeks. A total of 0.1 g of rice leaf tissue was crushed using liquid nitrogen. Total DNA isolation was performed using the protocol of the Wizard Genomic DNA Purification Kit.

Table 1. Specific primary characteristics for detecting blast resistance gene

| No. | Gene | Primer Sequence (Forward/Reverse) | Chromosomes number | Size (bp) | T Annealing (°C) | Reference |
|-----|--------------|--|--------------------|-----------|------------------|------------------------------|
| 1 | <i>Pib</i> | F:GACTCGGTGACCAATTGCC R:ATCAGGCCAGGCCAGATTG | 2 | 388 | 54°C | Yan <i>et al.</i> (2017) |
| 2 | <i>Pup1</i> | F:TCAAAATTCTTCAGGTATGTAC TCC R:TTGGGTGATCAGCTTCAGA | 12 | 1010 | 58°C | Heuer <i>et al.</i> (2009) |
| 3 | <i>Pita2</i> | F:AGCAGGTTATAAGCTAGGCC R:CTACCAACAAGTTCATAAA | 12 | 1042 | 59°C | Yan <i>et al.</i> , 2017 |
| 4 | <i>Pik</i> | F:CGTGCTGTCGCCCTGAATCTG R:CACGAACAAGAGTGTGCGG | 11 | 226 | 55 °C | Hayashi <i>et al.</i> (2006) |
| 5 | <i>Pii</i> | F:GGATGATGTGATCTGCAGAG R:CTCTGGTGTCTTGTAC | 9 | 484 | 56 °C | Yi <i>et al.</i> 2004, |

The crushed leaf powder was transferred into a 2-mL Eppendorf tube, followed by the addition of 600 µL of Nuclei Lysis Solution was added. The mixture was vortexed for 1–3 s and then heated for 15 minutes in a water bath at 65°C. Subsequently, 3 µL of RNase solution was added, and the sample was incubated at 37°C for 15 min. Next, 200 µL of protein precipitation solution was added, and the sample was centrifuged at 13,000 rpm for 3 min.

The supernatant was carefully transferred to a 1.5 mL microtube, followed by the addition of 600 µL of room-temperature isopropanol was added. The sample was centrifuged again at room temperature for 1 min. The supernatant was discarded, and the DNA pellet was air-dried for 15 min. Finally, 100 µL of DNA Rehydration Solution was added, and the sample was incubated at 65°C for 1 h or stored overnight at 4°C.

Total isolated DNA was used as a template for gene amplification in a polymerase chain reaction (PCR) machine using five specific primers for blast resistance genes. A 100 bp DNA ladder was used as a marker. The PCR reaction was carried out in a 10 µL reaction volume, consisting of 0.01 U/µL Phusion® DNA polymerase 100 mM dNTPs, 0.4 mM forward primer, 0.4 mM reverse primer, 0.5 µL template DNA.

The amplification process involved a series of thermal cycles as follows: an initial pre-denaturation step of 5 min at 94°C, followed by 35 cycles consisting of

denaturation at 94°C for 1 min, annealing at a specific temperature for 2 min, and extension at 72°C for 2 min, culminating in a final extension step of 10 min at 72°C. The success of the amplification process was evaluated via DNA electrophoresis using a 1.5% TBE agarose gel containing 0.6 g of agarose in 40 mL of 1× TAE buffer. The gel was then subjected to an electric current of 50 V for 30 min. Following electrophoresis, the gel was stained with Biotium GelRed® Nucleic Acid Gel Stain (10,000× dilution) and visualized using an LED transilluminator.

Results

Exploration of Bengkulu local rice varieties

The exploration of local varieties used a direct survey method to rice centers in four regencies in Bengkulu province, namely Central Bengkulu Regency, North Bengkulu Regency (Muko-muko), Kepahyang Regency, and Rejang Lebong Regency (Figure 1, Table 2). Exploration focused on rice varieties that are always grown by local farmers. The seeds of the local varieties were obtained from farmers, extension workers, and the local agriculture office.

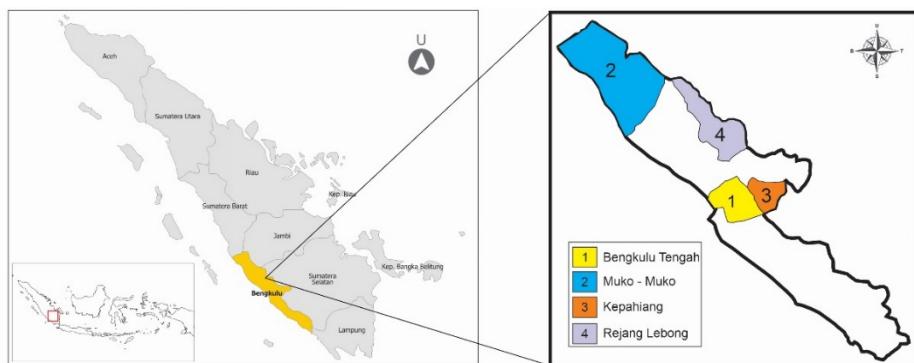


Figure 1. Exploration of local varieties in four regencies in Bengkulu province, namely Central Bengkulu Regency, North Bengkulu Regency (Muko-muko), Kepahyang Regency, and Rejang Lebong Regency

Table 2. Local varieties from four regencies in Bengkulu province, namely Central Bengkulu Regency, North Bengkulu Regency (Muko-muko), Kepahyang Regency and Rejang Lebong Regency

| Landrace | Coordinate | Location | District |
|----------|------------------------------|---------------------------------------|----------|
| Awi | 2°26'02.8"S 101°10'12.9"E | Desa Ranah Karya. Kec Lubuk Pinang | Mukomuko |
| | 2°26'02.8"S 101°10'12.9"E | Desa Ranah Karya. Kec Lubuk Pinang | |
| Siam | 2°25'41.8"S 101°09'49.5"E | Desa Ranah Karya. Kec Lubuk Pinang | Mukomuko |
| | 2°25'41.8"S 101°09'49.5"E | Desa Ranah Karya. Kec Lubuk Pinang | |

| Landrace | Coordinate | Location | District |
|----------------|----------------------|-----------------------------------|----------|
| | 2°29'59.2"S | | |
| Bulek | 101°06'05.4"E | Rawa Mulya SP 7. Kec XIV Koto | Mukomuko |
| | 2°28'34.4"S | | |
| Koreng | 101°06'23.4"E | Lubuk Sanai. Kec XIV koto | Mukomuko |
| | 2°31'44.2"S | Desa Sungai Ipuh. Kec Selagan | |
| Selagan | 101°19'28.2"E | Raya | Mukomuko |
| | 2°26'33.9"S | | |
| Mujiran | 101°08'53.9"E | Desa Arah Tiga. Kec Lubuk Pinang | Mukomuko |
| | 2°31'44.2"S | Desa Sungai Ipuh. Kec Selagan | |
| Suah ruput | 101°19'28.2"E | Raya | Mukomuko |
| | 2°30'22.3"S | Desa Sidomakmur Kec.Air | |
| Pulut barih | 101°11'16.4"E | Manjunto | Mukomuko |
| | 2°30'03.2"S | | |
| Pulut putih | 101°06'03.5"E | Dusun Baru Pelokan. Kec XIV Koto | Mukomuko |
| | 3.664494°S.102.48414 | Desa Penembang. Kec. Merigi | Bengkulu |
| Batu Bara | 0"E | Kelindang | Tengah |
| | 3.694467°S.102.49882 | Desa Datar Lebar. Kec. Taba | Bengkulu |
| Pelita | 3"E | Penanjung | Tengah |
| | 3.663295°S.102.48487 | | Bengkulu |
| Pundung | 1"E | Desa Jambu. Kec. Merigi Kelindang | Tengah |
| | 3.646704°S.102.47337 | | Bengkulu |
| Unggul | 8"E | Desa Jambu. Kec. Merigi Kelindang | Tengah |
| | 3.635071°S.102.37259 | | Bengkulu |
| Halus | 1"E | Desa Pagar Jati. Kec. Pagar jati | Tengah |
| | 3.664494°S.102.48414 | | Bengkulu |
| Gundung Putih | 0"E | Desa Jambu. Kec. Merigi Kelindang | Tengah |
| | 3.635071°S.102.37259 | | Bengkulu |
| Kuning | 1"E | Desa Penembang. Kec. Pagar jati | Tengah |
| | 3.694639°S.102.49898 | Desa Datar Lebar. Kec. Taba | Bengkulu |
| Rajo Lele | 3"E | Penanjung | Tengah |
| | 3.710074°S.102.52349 | Desa Rindu hati. Kec. Taba | Bengkulu |
| Silih Berganti | 8"E | Penanjung | Tengah |
| | 3.663918°S.102.48605 | | Bengkulu |
| Jurai-Jurai | 6"E | Desa Jambu. Kec. Merigi Kelindang | Tengah |

| Landrace | Coordinate | Location | District |
|---------------|---------------|-----------------------------------|-----------|
| | 3°29'22.9"S | Desa Lubuk Ubar. Kec. Curup | Rejang |
| Harum | 102°30'12.9"E | Selatan | Lebong |
| | 3°23'53.3"S | Desa Cawang Lama. Kec. Selupu | Rejang |
| Koreng Rendah | 102°35'30.1"E | Rejang | Lebong |
| | 3°24'27.0"S | Desa Cawang Baru. Kec. Selupu | Rejang |
| Koreng Tinggi | 102°35'41.6"E | Rejang | Lebong |
| | 3°22'48.4"S | | Rejang |
| Segumai | 102°26'41.5"E | Desa Purwodadi. Kec. Bermani Ulu | Lebong |
| | 3°21'29.1"S | Desa Air Bening. Kec. Bermani Ulu | Rejang |
| Puso | 102°26'54.4"E | Raya | Lebong |
| | 3°21'35.5"S | Desa Air Bening. Kec. Bermani Ulu | Rejang |
| Batik | 102°26'34.9"E | Raya | Lebong |
| Koreng | 3°40'45.6"S | Desa Penanjung Panjang. Kec. | |
| Kepahiang | 102°39'20.9"E | Tebat Karai | Kepahiang |
| | 3°35'33.3"S | | |
| Cempo | 102°38'54.4"E | Desa Suka Sari. Kec. Kabawetan | Kepahiang |
| | 3°34'03.6"S | | |
| Tumbar | 102°37'37.0"E | Desa Mekar Sari. Kec. Kabawetan | Kepahiang |
| | 3°38'57.2"S | Desa Suka Merindu. Kec. | |
| Pulut | 102°36'50.5"E | Kepahiang | Kepahiang |

Agronomic characteristics of local varieties

The agromorphological characteristics of the 30 rice varieties studied as shown in Table 3. The mean values and standard deviations provided an overview of the Bengkulu local varieties' agromorphological characteristics. A heat map of the Pearson correlation matrix across the different characteristics of Bengkulu local varieties is shown in Figure 2, while a dendrogram of 30 local rice accessions from Bengkulu was constructed in Figure 3.

Table 3. Agro-morphological characteristics of 30 local rice varieties from four regencies in Bengkulu Province, namely Central Bengkulu Regency, North Bengkulu Regency (Muko-Muko), Kepahyang Regency, and Rejang Lebong Regency

| Landraces | X ± SD (Mean ± Standard Deviation) | | | | | | | | | | | |
|-------------|------------------------------------|----------|--------|--------|---------|-----------|-----------|------------|------------|---------|------------|------------|
| | PH* | TNT | NPT | PL | FL | DF | DM | TNG | NFG | NEG | GWH | 1.000 GW |
| Awi | 111.6 ± | | 19.3 ± | 22.5 ± | 42.9 ± | 94.8 ± | 121.2 ± | | | 8.8 ± | 97.9 ± | 26.16 ± |
| | 8.7 | 26 ± 3.8 | 4.2 | 1.0 | 1.8 | 0.4 | 0.4 | 99.3 ± 4.5 | 90.1 ± 3.2 | 2.51 | 20.9 | 0.1 |
| Siam | 107.0 ± | 30.1 ± | 20.3 ± | 23.4 ± | 33.6 ± | 91.5 ± | 118.8 ± | | | 10.3 ± | | |
| | 6.7 | 3.1 | 2.9 | 0.9 | 1.6 | 0.5 | 0.4 | 107 ± 8.2 | 91.3 ± 6.0 | 4.6 | 97.8 ± 6.3 | 29.2 ± 0.2 |
| Lebek | 27.4 ± 18.7 ± | | 23.7 ± | 39.3 ± | 93.2 ± | 121.2 ± | 135.5 ± | 107.8 ± | 18.8 ± | 96.5 ± | | |
| | 97.8 ± 5.9 | 3.1 | 2.9 | 0.9 | 2.5 | 0.4 | 0.4 | 5.4 | 5.7 | 5.2 | 17.5 | 25.2 ± 0.1 |
| Bulek | 36.1 ± | 21.8 ± | 23.0 ± | 35.2 ± | 90.2 ± | 119.1 ± | 136.9 ± | 112.4 ± | 16.8 ± | | | 22.97 ± |
| | 94.7 ± 3.8 | 3.1 | 2.6 | 0.8 | 1.7 | 0.4 | 0.3 | 6.1 | 21.8 | 8.6 | 77.7 ± 9.5 | 0.1 |
| Koreng | 119.9 ± | 27.2 ± | 18.7 ± | 22.8 ± | 43.2 ± | 100.4 ± | 124.4 ± | 101.2 ± | | 11.1 ± | 93.7 ± | 23.14 ± |
| | 10.5 | 4.5 | 3.3 | 0.9 | 1.2 | 0.5 | 0.5 | 5.7 | 88.8 ± 7.9 | 3.3 | 19.0 | 0.1 |
| Selagan | 19.9 ± | 12.8 ± | 23.7 ± | 42.0 ± | 101.4 ± | 124.2 ± | 104.8 ± | | | 12.6 ± | 78.0 ± | 26.95 ± |
| | 95.7 ± 9.5 | 2.1 | 0.8 | 1.2 | 0.8 | 0.8 | 0.4 | 9.8 | 92.2 ± 7.9 | 4.7 | 15.4 | 0.1 |
| Mujiran | 100.2 ± | 27.4 ± | 21.3 ± | 23.5 ± | 45.3 ± | 89.6 ± | 117.6 ± | 111.2 ± | | 14.3 ± | 97.4 ± | 26.49 ± |
| | 7.6 | 3.8 | 4.3 | 0.8 | 0.9 | 0.8 | 0.8 | 7.6 | 96.9 ± 6.9 | 4.8 | 18.9 | 0.1 |
| Suah Ruput | 28.1 ± | 21.9 ± | 22.7 ± | 33.2 ± | 90.2 ± | 120.2 ± | 138.3 ± | 113.7 ± | 20.4 ± | 109.1 ± | | 25.78 ± |
| | 94.3 ± 5.5 | 4.6 | 4.5 | 0.8 | 1.8 | 0.4 | 0.4 | 6.4 | 5.7 | 3.1 | 11.4 | 0.1 |
| Pulut Barih | 120.5 ± | 18.0 ± | 13.5 ± | 24.3 ± | 35.6 ± | 91.6 ± | 122.6 ± | 100.1 ± | | 9.4 ± | 88.1 ± | |
| | 3.8 | 2.1 | 0.9 | 0.8 | 1.3 | 0.5 | 0.5 | 5.3 | 90.2 ± 6.2 | 1.82 | 17.4 | 29.1 ± 0.1 |
| Pulut Putih | 30.4 ± | 24.7 ± | 26.1 ± | 34.5 ± | 90.3 ± | 120.3 ± | 101.8 ± | | | 10.3 ± | 101.1 ± | |
| | 88.6 ± 2.7 | 3.3 | 2.9 | 1.2 | 2.3 | 0.5 | 0.5 | 7.2 | 91.4 ± 5.9 | 2.6 | 18.8 | 28.0 ± 0.1 |
| Batu Bara | 28.4 ± | 22.5 ± | 19.2 ± | 33.4 ± | 90.8 ± | 120.3 ± | 93.4 ± | 87.6 ± | 5.7 ± | 66.8 | | 28.99 ± |
| | 73.6 ± 5.1 | 3.6 | 2.6 | 3.4 | 6.3 | 1.0 | 0.5 | 12.9 | 12.6 | 0.89 | ±12.7 | 0.03 |
| Pelita | 30.7 | 23.9 ± | 18.9 ± | 34.2 ± | 87.2 ± | 120.6 ± | 108.2 ± | 102.3 ± | 5.9 ± | | | 27.67 ± |
| | 85.1 ± 5.6 | ±4.2 | 3.4 | 2.7 | 4.9 | 1.5 | 0.5 | 16.0 | 15.5 | 1.7 | 79.0 ± 7.9 | 0.04 |
| Pundung | 33.1 ± | 22.9 ± | 22.5 ± | 31.2 ± | | | 127.3 ± | 121.7 | 5.6 ± | | | 23.25 ± |
| | 80.8 ± 5.5 | 1.6 | 2.5 | 2.4 | 4.6 | 86 ± 0.66 | 121 ± 0.5 | 16.6 | ±15.7 | 2.34 | 72.2 ± 6.5 | 0.13 |
| Unggul | 28.7 ± | 20.9 ± | 21.7 ± | 35.7 ± | | | 117.7 ± | 111.3 ± | 6.4 ± | 67.8 ± | | 25.71 ± |
| | 80.6 ± 6.2 | 3.6 | 2.4 | 2.2 | 4.0 | 90 ± 0.6 | 122 ± 0.7 | 10.1 | 10.3 | 1.74 | 10.8 | 0.08 |
| Halus | 82.7 ± | 12.7 ± | 6.00 ± | 21.1 ± | 41.8 ± | | 123.8 ± | 164.5 ± | 155.9 ± | 8.5 ± | | 20.16 ± |
| | 11.5 | 2.7 | 1.5 | 1.7 | 1.1 | 104 ± 1.3 | 1.3 | 14.4 | 14.5 | 1.41 | 22.2 ± 2.9 | 0.06 |

| Landraces | PH* | X ± SD (Mean ± Standard Deviation) | | | | | | | | | | |
|----------------|----------------|------------------------------------|---------------|---------------|----------------|-----------------|------------|-----------------|----------------|----------------|--------------------|----------------|
| | | TNT | NPT | PL | FL | DF | DM | TNG | NFG | NEG | GWH | 1.000 GW |
| Gundung Putih | 80.9 ± 4.0 | 29.4 ± 2.5 | 24.2 ± 2.8 | 21.3 ± 1.6 | 34.7 ± 4.3 | 83 ± 0.6 | 1.1 | 121.7 ± 24.3 | 131.0 ± 24.5 | 119.1 ± 1.7 | 72.9 ± 12.9 | 22.37 ± 0.04 |
| | 119.8 ± 9.00 ± | 5.00 ± 9.00 ± | 24.1 ± 5.00 ± | 36.5 ± 24.1 ± | 101.7 ± 36.5 ± | 124.4 ± 101.7 ± | 0.8 | 212.2 ± 124.4 ± | 198.9 ± 15.9 | 13.3 ± 18.4 | 29.5 ± 4.4 | 25.83 ± 10.0 |
| Kuning | 10.2 | 1.5 | 1.7 | 1.6 | 3.6 | 0.7 | 0.8 | 15.9 | 18.4 | 4.4 | 10.0 | 0.04 |
| | 29.1 ± 10.2 | 24.1 ± 1.5 | 19.7 ± 1.7 | 27.8 ± 1.6 | 87.2 ± 3.6 | 121.5 ± 0.7 | 1.1 | 100.6 ± 121.5 ± | 88.8 ± 19.3 | 11.7 ± 17.4 | 73.3 ± 2.6 | 27.02 ± 11.6 |
| Rajo Lele | 79.1 ± 5.2 | 3.6 | 2.9 | 1.0 | 2.4 | 0.4 | 1.1 | 116.3 ± 78.2 ± | 106.2 ± 19.3 | 10.1 ± 116.3 ± | 11.6 | 0.06 |
| | 26.2 ± 79.1 ± | 21.9 ± 3.6 | 18.6 ± 2.9 | 24.7 ± 1.1 | 78.2 ± 3.6 | 121 ± 0.4 | 1.3 | 6.2 | 6.9 | 3.1 | 17.71 ± 51.3 ± 6.8 | 0.03 |
| Silih Berganti | 72.5 ± 5.4 | 3.2 | 2.9 | 1.1 | 3.6 | 0.4 | 1.21 ± 1.3 | 111.3 ± 6.2 | 11.4 ± 6.9 | 76.3 ± 3.1 | 27.8 ± 51.3 ± 6.8 | 0.03 |
| | 26.6 ± 72.5 ± | 21.6 ± 3.2 | 19.8 ± 2.9 | 28.5 ± 1.1 | 89.8 ± 3.6 | 123.2 ± 0.4 | 1.3 | 111.3 ± 123.2 ± | 99.9 ± 111.3 ± | 14.4 ± 11.4 ± | 76.3 ± 11.4 ± | 27.8 ± 76.3 ± |
| Jurai-Jurai | 70.8 ± 5.9 | 3.3 | 3.9 | 0.6 | 2.0 | 1.0 | 1.4 | 7.4 | 99.9 ± 6.5 | 3.2 | 14.4 | 0.03 |
| | 104.7 ± 70.8 ± | 24.0 ± 3.3 | 20.3 ± 3.9 | 24.8 ± 0.6 | 33.8 ± 2.0 | 121.4 ± 1.0 | 1.4 | 101.7 ± 121.4 ± | 9.4 ± 7.4 | 103.8 ± 9.4 ± | 24.04 ± 103.8 ± | 24.04 ± 14.4 |
| Harum | 4.7 | 3.1 | 4.1 | 1.1 | 2.4 | 94.7 ± 0.5 | 0.7 | 10.3 | 92.2 ± 8.5 | 3.23 | 17.7 | 0.10 |
| | 100.9 ± 4.7 | 29.7 ± 3.1 | 22.5 ± 4.1 | 21.9 ± 1.1 | 43.9 ± 2.4 | 100.3 ± 94.7 ± | 0.5 | 130.1 ± 0.7 | 102.2 ± 10.3 | 8.9 ± 92.2 ± | 100.9 ± 8.9 ± | 20.7 ± 100.9 ± |
| Koreng Rendah | 4.8 | 3.8 | 4.3 | 0.8 | 0.9 | 0.5 | 0.6 | 14.0 | 11.0 | 3.3 | 10.4 | 0.24 |
| | 111.3 ± 4.8 | 29.5 ± 3.8 | 22.9 ± 4.3 | 22.6 ± 0.8 | 37.2 ± 0.9 | 100.8 ± 0.5 | 1.2 | 131.6 ± 14.0 | 126.7 ± 11.0 | 13.8 ± 11.0 | 103.5 ± 12.1 | 10.4 ± 12.1 |
| Koreng Tinggi | 7.7 | 4.3 | 2.6 | 0.5 | 3.5 | 1.2 | 2.2 | 13.6 | 12.1 | 2.7 | 7.4 | 20.9 ± 0.2 |
| | 28.2 ± 7.7 | 22.1 ± 4.3 | 21.6 ± 2.6 | 21.6 ± 0.5 | 100.7 ± 3.5 | 130.6 ± 1.2 | 1.2 | 131.9 ± 13.6 | 131.9 ± 12.1 | 16.4 ± 12.1 | 100.2 ± 16.4 ± | 22.2 ± 100.2 ± |
| Segumai | 98.1 ± 1.7 | 3.6 | 4.2 | 0.5 | 0.5 | 1.2 | 0.5 | 9.2 | 9.2 | 3.2 | 2.9 | 0.07 |
| | 111.7 ± 98.1 ± | 30.5 ± 3.6 | 22.8 ± 4.2 | 22.5 ± 0.5 | 36.7 ± 0.5 | 104.2 ± 1.2 | 1.2 | 134.4 ± 9.2 | 131.9 ± 9.2 | 8.03 ± 9.2 | 103.4 ± 8.03 ± | 23.8 ± 103.4 ± |
| Puso | 6.7 | 4.6 | 3.2 | 0.9 | 1.7 | 1.0 | 0.9 | 91.8 ± 7.4 | 83.9 ± 6.4 | 1.9 | 8.1 | 1.93 |
| | 103.5 ± 6.7 | 23.2 ± 4.6 | 18.8 ± 3.2 | 22.2 ± 0.9 | 35.5 ± 1.7 | 109.2 ± 1.0 | 1.2 | 139.3 ± 91.8 ± | 102.5 ± 83.9 ± | 8.2 ± 83.9 ± | 104.1 ± 8.2 ± | 26.6 ± 104.1 ± |
| Batik | 7.6 | 2.6 | 2.7 | 0.7 | 1.3 | 0.9 | 0.8 | 5.7 | 94.1 ± 4.4 | 2.3 | 7.1 | 0.04 |
| | 102.8 ± 7.6 | 44.8 ± 2.6 | 38.4 ± 2.7 | 22.5 ± 0.7 | 33.2 ± 1.3 | 94.1 ± 0.9 | 1.2 | 120.1 ± 5.7 | 159.6 ± 94.1 ± | 10.8 ± 159.6 ± | 114.9 ± 10.8 ± | 20.6 ± 114.9 ± |
| Kepahiang | 10.1 | 6.0 | 7.6 | 0.9 | 0.4 | 1.4 | 1.2 | 15.8 | 15.2 | 2.8 | 9.1 | 20.6 ± 0.3 |
| | 111.6 ± 10.1 | 37.2 ± 6.0 | 27.9 ± 7.6 | 19.7 ± 0.9 | 27.8 ± 0.4 | 90.4 ± 1.4 | 1.2 | 210.4 ± 15.8 | 108.8 ± 15.2 | 9.03 ± 108.8 ± | 108.3 ± 9.03 ± | 28.9 ± 108.3 ± |
| Cempo | 8.6 | 5.1 | 3.9 | 1.0 | 3.7 | 0.5 | 0.5 | 12.9 | 11.7 | 1.7 | 8.3 | 28.9 ± 0.1 |
| | 122.1 ± 8.6 | 28.8 ± 5.1 | 23.3 ± 3.9 | 23.6 ± 1.0 | 31.6 ± 3.7 | 91.4 ± 0.5 | 1.2 | 120.8 ± 12.9 | 108.6 ± 11.7 | 10.5 ± 108.6 ± | 102.1 ± 10.5 ± | 28.8 ± 102.1 ± |
| Tumbar | 8.5 | 2.9 | 2.4 | 0.4 | 2.0 | 0.51 | 0.78 | 8.1 | 98.3 ± 6.9 | 1.6 | 6.7 | 28.8 ± 0.1 |
| | 37.5 ± 8.5 | 23.4 ± 2.9 | 20.8 ± 2.4 | 39.5 ± 0.4 | 121.6 ± 2.0 | 91 ± 0.51 | 1.83 | 121.6 ± 8.1 | 92 ± 3.4 | 8.2 ± 98.3 ± | 26.4 ± 1.6 | 28.8 ± 1.6 |
| Pulut | 93.4 ± 6.1 | 6.4 | 3.8 | 0.7 | 1.3 | 91 ± 1.15 | 1.83 | 92 ± 3.4 | 83.6 ± 2.8 | 1.65 | 99.6 ± 5.7 | 26.4 ± 0.3 |

*PH = Plant Height; TNT = Total number of Tiller; NPT = Number of productive tiller; PL = Panicle Length; FL = Flag length DF = Day flowering; DM = Day maturity; TNG = Total Grain per hill; NFG = Number of fill grain; NEG = Number of empty grain; 1000 WG = Weight of 1000 grain; GWH = Grain weight per hill

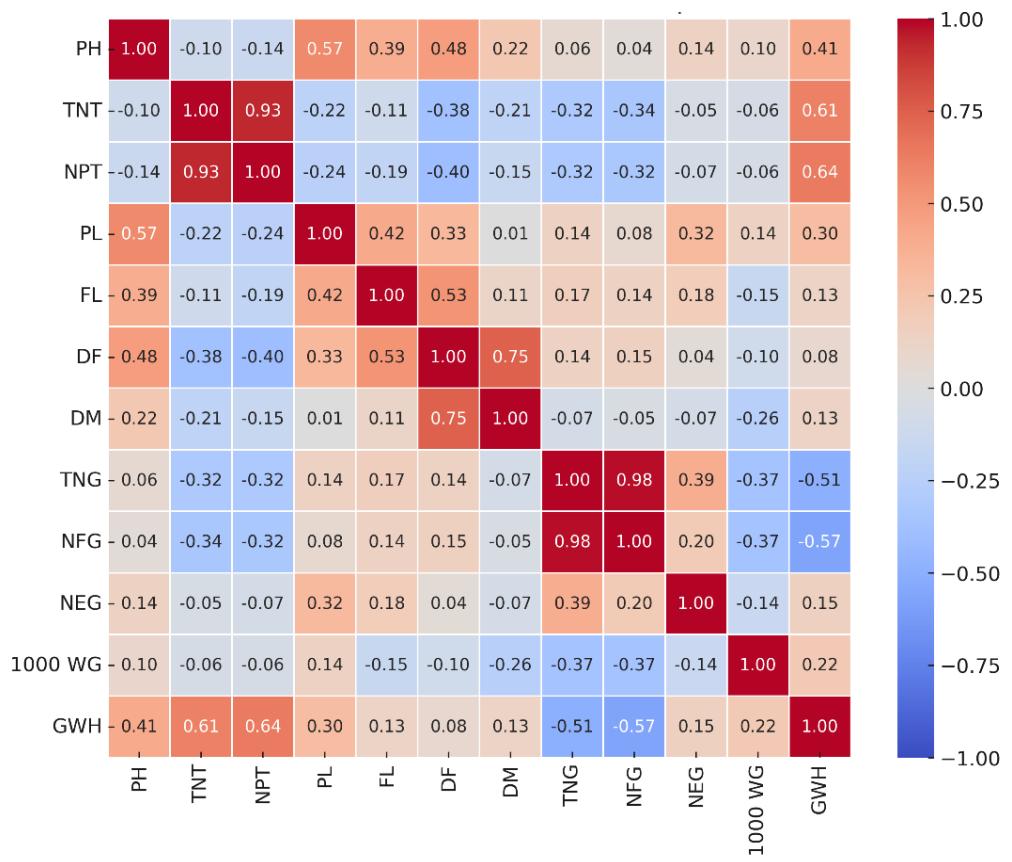


Figure 2. Heat map of the Pearson correlation matrix across the different characteristics of Bengkulu local varieties, the color gradient indicates highlighting strong positive correlations (red) and strong negative correlations (blue). PH = Plant Height; TNT = Total number of Tiller; NPT = Number of productive tiller; PL = Panicle Length; FL = Flag length DF = Day flowering; DM = Day maturity; TNG = Total Grain per hill; NFG = Number of fill grain; NEG = Number of empty grain; 1000 WG = Weight of 1000 grain; GWH = Grain weight per hill

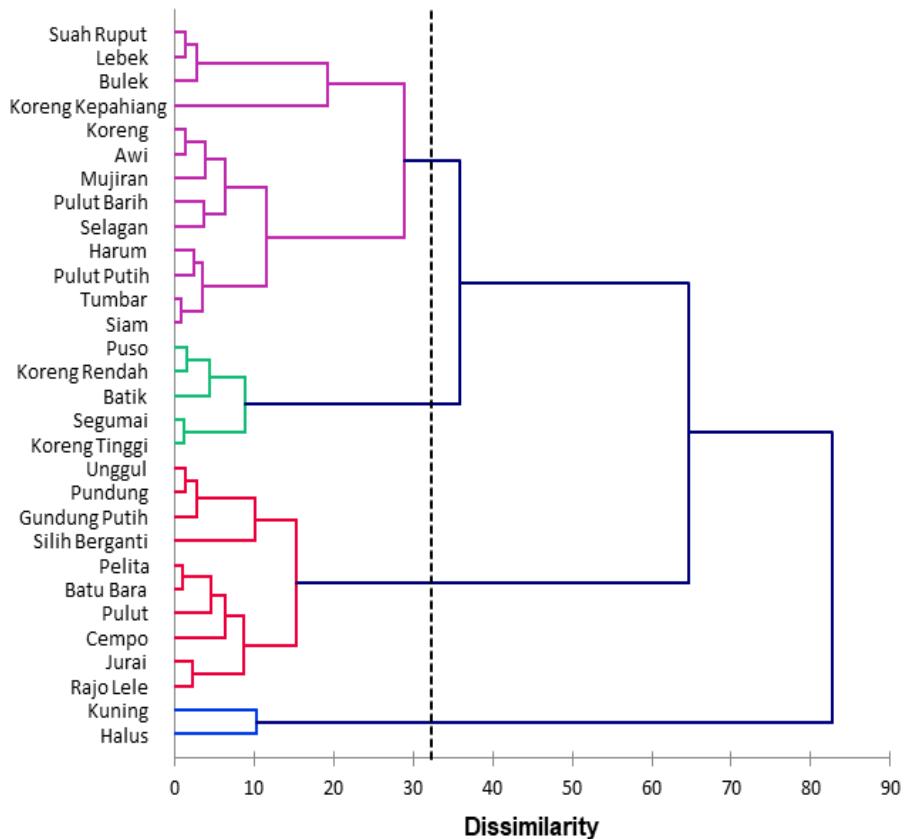


Figure 3. A dendrogram of 30 local rice accessions from Bengkulu was constructed based on agro-morphological characteristics.

Identification of blast resistance genes

The amplification of blast resistance genes (*Pup1*, *Pita-2*, *Pii*, *Pib*, and *Pik*) in 30 local rice varieties are shown in Figure 4, 5.

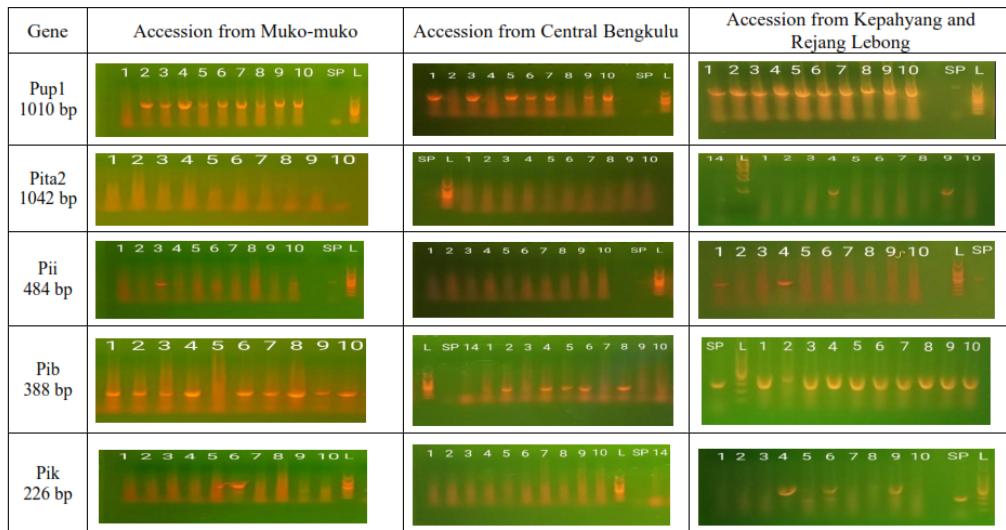


Figure 4. PCR amplification for blast resistance genes using specific primers *Pup1*, *Pita-2*, *Pii*, *Pib*, and *Pik* (L = Marker 100 bp, SP = Situ Patenggang; accession from Muko-muko: 1. Awi, 2. Siam, 3. Lebek, 4. Bulek, 5. Koreng, 6. Selagan, 7. Mujiran, 8. Suah Ruput, 9. Pulut Barih, 10. Pulut Putih; accession from central Bengkulu: 1. Batubara, 2. Pelita, 3. Pundung, 4. Unggul, 5. Halus, 6. Gundung Putih, 7. Kuning, 8. Rajo Lele, 9. Silih Berganti, 10. Jurai-jurai; accession from Kepahyang and Rejang Lebong: 1. Harum, 2. Koreng Rendah, 3. Koreng Tinggi, 4. Segumai, 5. Puso, 6. Batik, 7. Koreng Kepahiang, 8. Cempo, 9. Tumbar, 10. Pulut)

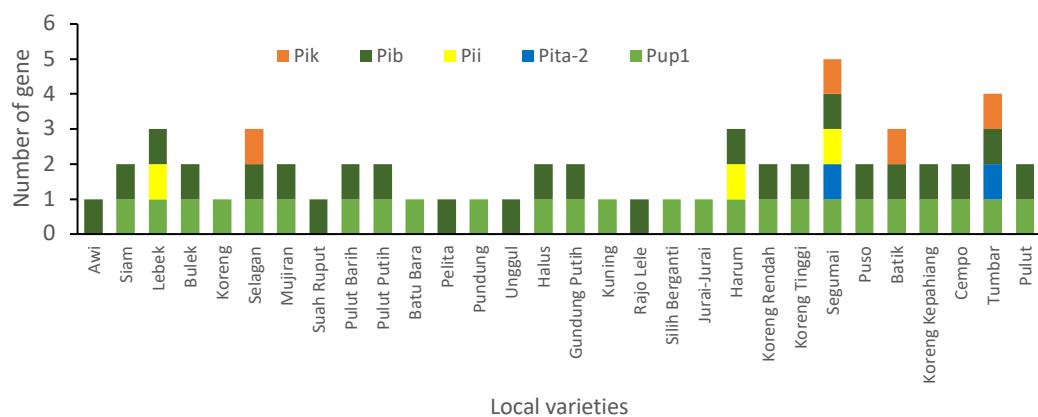


Figure 5. The presence of blast resistance genes in local rice varieties (*Pup1*, *Pita-2*, *Pii*, *Pib*, and *Pik*). Several varieties possess multigenic resistance, which has the potential to overcome the diversity of blast races in the field

Discussion

The exploration obtained 30 local varieties each from four regencies in Bengkulu province, namely, Central Bengkulu Regency, North Bengkulu Regency (Muko-muko), Kepahyang Regency, and Rejang Lebong Regency. Farmers continue to cultivate these local rice varieties in 23 villages. These results indicated that these varieties are well adapted to local conditions and continue to play an essential role in traditional farming systems. Its potential for breeding programs to improve traits such as yield, resistance, and adaptability (Huang *et al.*, 2022; Khannetah *et al.*, 2021).

The agromorphology of the 30 local rice varieties, plant height ranged from 70.8 cm (Jurai-Jurai) to 122.1 cm (Tumbar). The tallest were Tumbar (122.1 cm) and Pulut Barih (120.5 cm), and the two shortest are Silih Berganti (72.5 cm) and Batu Bara (73.6 cm). Increased plant height can increase competitiveness against weeds, but it is sensitive to lodging (Wu *et al.*, 2022). The total number of tillers ranged from 9.0 (Kuning) to 44.8 (Koreng Kepahiang). Varieties with the highest total number of tillers, such as Koreng Kepahiang (44.8) and Pulut (37.5), showed higher yield potential. The number of productive tillers ranged from 5.0 (Yellow) to 38.4 (Koreng Kepahiang). Varieties with a high number of productive tillers, such as Koreng Kepahiang (38.4) and Pulut (23.4), produced more panicles, which had the potential to produce a large amount of grain (Takai, 2024).

The panicle length ranged from 18.6 cm (Silih Berganti) to 26.1 cm (Pulut Putih). Cultivars with longer panicles such as Pulut Putih (26.1 cm) and Harum (24.8 cm) had the potential to produce more grain per panicle (Parida *et al.*, 2022). The flowering time (DF – Days to Flowering) varied from 78.2 days (Silih Berganti) to 109.2 days (Batik), while maturity time varied from 117.6 days (Mujiran) to 139.3 days (Batik). Varieties that had a longer flowering and maturity period such as Batik (DF: 109.2, DM: 139.3) had the potential for higher yields, but are also more vulnerable to climate change. Rice was based on the harvest time into several group: aged (more than 151 days after sowing; medium age 125-150) days after sowing; early age (105-124 days after sowing); very early age (90-104 days after sowing); and ultra-early (< 90 days after sowing) (Indonesia Rice Research Centre, 2017).

The average number of grains was between 91.8 (Puso) and 212.2 (Kuning). The number of filled grains (NFG) ranged between 83.6 (Pulut) and 198.9 (Kuning), while the number of empty grains (NEG) from 5.6 (Pundung) to 20.4 (Suah Ruput). Total number of filled grains were highest in the Kuning variety, reflecting greater yield potentials. Silih Berganti and Pundung had fewer grains that were not filled; each plant can fill more grains. Research by Zhou *et*

al. (2018), Herawati *et al.* (2021a), and Parida *et al.* (2022) found that the number of grains per panicle is a key factor in determining rice yield and a crucial consideration for breeders when developing high-yielding varieties. 1000-grain weight (1000 GW) ranged from 17.71 g (Silih Berganti) to 29.2 g (Siam). Generally, varieties with larger seed sizes will fall off easily and are more prone to breakage during post-harvest handling.

Several key agronomic traits showed strong positive correlations ($r > 0.5$), indicating important relationships in rice growth and productivity (Figure 2). A high correlation coefficient of $r = 0.931$ between the total number of tillers and the number of productive tillers suggested that as the total number of tillers increased. The total number of grains per hill (TNG) and the number of filled grains (NFG) was correlated of 0.978. Increasing rice yield involved using the number of productive tillers per hill as a key factor in selecting more productive varieties. At least 330 productive tillers per m² (10-14 plants per hill) are required to boost paddy rice yields by 10% (Peng *et al.*, 2008).

There was a strong correlation of 0.749 between days to flowering (DF) and days to maturity (DM), with rice varieties that took longer to flower also taking longer to mature, indicating a significant relationship between these two growth stages. Plant height (PH) and panicle length (PL) were correlated at 0.573, indicating that taller plants generally produced longer panicles, suggesting that plant height can increase the ability to develop rice grains. Long panicles allow the formation of many grains, but insufficient photosynthate supply from the leaves will increase the number of empty grains. According to Kobata and Iida (2004), the occurrence of empty grain can be attributed to the low efficiency of assimilate distribution from leaves to grain. According to Khush (2013), subpar grain filling was caused by insufficient apical dominance in the panicle, a tightly packed arrangement of spikelets and a small number of large vascular bundles limiting assimilate transport to the grain.

Research finding found that there was a correlation of 0.637 between grain weight per hill (GWH) and number of productive tillers (NPT), suggesting that tillers developed from multiple buds per plant showed a positive impact on yield since those producing panicles tend to result in greater grain weight per hill. The grain weight hill-1 (GWH) and total number of tillers (TNT) correlation was 0.612. Higher grain weight is generally associated with more tillers, although grain filling efficiency and nutrient availability can also impact overall productivity (Parida *et al.*, 2022).

Dendograms illustrated the genetic connections among the accessions by clustering them according to their differences in agro-morphological characteristics. This clustering analysis facilitated the identification of closely related varieties and provides valuable insights into their diversity, which is

crucial for breeding and conservation efforts. A dendrogram grouped 30 local rice varieties into four clusters based on their agronomic and morphological characteristics as derived from a similarities matrix. Cluster 1 comprised 13 varieties, which were Awi Siam, Lebek, Bulek, Koreng, Selagan, Mujiran, Suah Ruput, Pulut Barih, Pulut Putih, Harum Koreng Kepahiang, and Tumbar. This cluster comprised 10 types: Batu Bara, Pelita, Pundung, Unggul, Gundung Putih, Rajo Lele, Silih Berganti, Jurai, Cempo and Pulut. Cluster 3 comprised only two varieties, Halus and Kuning. Cluster 4 comprised five varieties, namely, Koreng Rendah, Koreng Tinggi, Segumai, Puso, and Batik. Each cluster demonstrated that these varieties are found to be more genetically or phenotypically similar to one another than to varieties in other clusters.

Molecular marker analysis results showed that *Pib* was the gene most often found in local varieties, present in 26 out of 30, whereas *Pup1* was common but not the case for most genetic gains, occurring in 18 out of 30 varieties. In contrast, the least common gene is *Pita2*, which is only found in a few varieties, implying its scarcity among the range of local rice varieties available. On the other hand, *Pik* is found to be a quite rare and found only in cultivars such as Harum, Batik and Pulut Putih.

The most resistant varieties, carrying multiple resistance genes, are found in Harum, Selagan, Lebek, and Batik, which is possesd three distinct resistance genes, namely *Pup1*, *Pib*, and *Pik*. This may offer to improve resistance against blast dIt appears that Segumai and Tumbar found to be e a wider resistance capability, featuring between four and five resistance genes (*Pup1*, *Pita2*, *Pii*, *Pik*, and *Pib*). Use of pyramiding genes in rice can help to combat a diverse and highly dynamic populations of blast pathogens in the field (Xiao *et al.*, 2015; Orasen *et al.*, 2020; Wang *et al.*, 2023).

Genes associated with virulence traits in *Pyricularia oryzae* have been genetically studied by several researchers (Xu *et al.*, 2014; Ramkumar *et al.*, 2015). Several blast resistance genes that control compatibility with certain varieties, and genes that control blast development during infection including genes that affect apresorium formation and apresorium penetration have also been reported by several researchers (Vasudevan *et al.*, 2016; Zheng *et al.*, 2016; Xiao *et al.*, 2017). Genes that have been successfully cloned are *Pi37* on chromosome 1 (Lin *et al.*, 2007), *Pib* on chromosome 2, *Pi9* (Qu *et al.*, 2006), *Pid2* (Chen *et al.*, 2006) on chromosome 6, and *Pita* on chromosome 12 (Bryan *et al.*, 2000). The *Pup1* gene shows the presence of dirigent-like genes with fatty acid α -dioxygenase, and aspartate proteininase that play an important role in producing proteins involved in lignin biosynthesis that affect cell wall hardness (Heuer *et al.*, 2009).

Conversely, the varieties with low resistance potential are found in Ungkul and Rajo Lele, which possess only one resistance gene (*Pib*). This may render them more vulnerable to the blast disease than varieties with a broader genetic resistance profile. Varieties with a single resistance gene typically provide protection only against specific virulent races. In contrast, previous studies have shown that the presence of multiple major resistance genes can confer long-term, broad-spectrum resistance (Xiao *et al.*, 2016; Li *et al.*, 2020). In addition, the distribution pattern of resistance genes showed that *Pib* and *Pup1* were the most prevalent, indicating that they are present in most local rice accessions. On the other hand, *Pik* and *Pita2* genes are rarely found, so it is necessary to increase the resistance of local varieties through introgression of these genes through breeding programs.

The high grain weight potential varieties are Koreng Kepahiang, Kuning, Suah Ruput, and Tumbar, which found a high total amount of productive tillers, a high total grain weight, and a relatively good 1000-grain weight. Short-growth duration varieties such as Silih Berganti, Pundung, and Pelita with lower DF and DM values are found to be suitable for regions with relatively short growing seasons. Pundung and Silih Berganti are types with a low empty grain percentage (high efficiency). Siam, Pulut Barih, and Pulut Putih are found to be the varieties with large and high-quality grains. The *Pib* and *Pup1* genes are responsible for the blast resistance of the majority of rice varieties. Varieties such as Harum and Batik, as well as the current ones Segumai and Tumbar, found a stronger combination of their resistance genes and are considered germplasm useful for breeding programs. In contrast, varieties with a lower number of resistance genes may need to improve their resistance by being crossed with varieties that possess a more complete set of blast-resistance genes.

Acknowledgments

This work was supported by the Faculty of Agriculture, University of Bengkulu through the PNBP Research Ungulan Program (Grant # 2982/UN30.11/PT/2024).

Conflicts of interest

The authors declare no conflict of interest.

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(Received: 14 July 2025, Revised: 26 December 2025, Accepted: 6 January 2026)