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Research Article

Evaluation of Agronomic Performance and Genetic Diversity in Indonesian Pigmented Rice Using SCoT (Start Codon Targeted) Markers

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ABSTRACT

Indonesia is rich in biodiversity, including rice (*Oryza sativa* L.), one of the most important and widely cultivated crops. However, the utilization of local Indonesian rice germplasm in breeding programs remains limited due to the lack of reliable characterization data. Therefore, it's essential to establish a reliable method to ensure variety protection by breeders. In addition, understanding the relationship between morphological traits and genetic diversity is crucial for a targeted breeding study aimed at evaluating genetic diversity among 12 local rice genotypes from Indonesia through agronomic traits and molecular analysis using start codon targeted (SCoT) markers. Statistical analyses, including cluster and diversity indices, were applied to interpret the data. Characterization was conducted based on the descriptors from The International Rice Research Institute (IRRI) and Bioversity International, while molecular analysis employed 23 selected SCoT primers. The effectiveness of the markers was assessed through polymorphism percentage, PIC values, and cluster analysis. A polymorphism level of 69% was observed, with PIC values ranging from 0.3610 to 0.4646, suggesting that the SCoT markers used provided adequate informativeness for diversity analysis. The study revealed high genetic variability, with heritable traits and informative SCoT markers, demonstrating their potential for pre-breeding and diversity analysis.

1. Introduction

Rice (*Oryza sativa* L.) is regarded as the most important cereal commodity, serving as a crucial food source for nearly half of the world's population Birla *et al.* 2017. In Indonesia, rice is a widely consumed staple food (Rozi *et al.* 2023), comprising various genotypes that exhibit diverse agronomic traits. Among these, the popularity of pigmented rice varieties is growing rapidly, driven by both their unique visual appeal and the health benefits associated with their anthocyanin content (Yi *et al.* 2020). This increasing consumer interest underscores the rising awareness of health-related considerations in food choices, making the diversity of rice varieties essential for ensuring food security and supporting national food diversification strategies (Mohidem *et al.* 2022).

Despite the growing interest, there are many underexplored, underscoring the urgent need for focused research and breeding programs to unlock their full potential (Sahu *et al.* 2023). Indonesia is a habitat to various genotypes, including Maja, Cicurug Maja, Kulur, Habang Separi, and Pare Lontong Tanduk, which have yet to be thoroughly characterized. Utilizing these genetic resources is critical for enhancing agricultural productivity and ensuring food security amid future challenges such as climate change, population growth,

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and the demand for sustainable agriculture (Lee *et al.* 2020).

Rice has evolved into one of the most diverse food crops and is cultivated in various regions around the world. This plant possesses a wide range of germplasm, which now plays a crucial role in increasing productivity (Ren *et al.* 2023). This research is part of a preliminary study aimed at exploring genetic diversity through the use of molecular markers. The application of marker-assisted technology is a promising approach to understanding this diversity. Molecular markers are considered more effective for revealing genetic diversity among rice varieties as they are non-destructive and unaffected by environmental factors (Choudhury *et al.* 2013). Researchers consistently use DNA fingerprinting techniques to assess the level of genetic variation within germplasm or cultivars, allowing them to be categorized into specific groups (Prasad *et al.* 2020).

Various molecular markers, such as Start Codon Targeted (SCoT), Simple Sequence Repeat (SSR), and Single Nucleotide Polymorphism (SNP), have been widely used in plant genetic studies, including rice. Among them, SCoT is valued for its simplicity, cost-effectiveness, and gene-targeted nature. As a dominant marker based on conserved sequences flanking the ATG start codon, it enables amplification of gene-associated regions without prior genomic data (Collard & Mackill 2009). This makes it suitable for non-model species and underutilized varieties (Omar *et al.* 2023). It generates high polymorphism and reproducible results, and because it targets coding-adjacent regions, it may indirectly reflect gene expression and regulation (Amiteye 2021; Rai 2023). These strengths support its application in gene mapping, diversity analysis, and marker-assisted selection, particularly in low-resource breeding programs. Numerous studies have successfully employed SCoT for genetic diversity analysis in crops such as sesame (Mesfer Alshamrani *et al.* 2022), chili (Gupta *et al.* 2019), and sugarcane (Que *et al.* 2014), confirming its wide applicability in crop research and improvement.

Building upon this foundation, this research aims to evaluate the genetic diversity of Indonesian pigmented rice varieties through morphological assessments and molecular analyses utilizing SCoT markers. By uncovering the genetic information associated with these varieties, the study intends to inform breeding programs, making them more efficient and targeted in developing superior pigmented rice cultivars. Ultimately, understanding both agronomic and molecular diversity is key to advancing plant breeding and ensuring the adaptability of rice to future agricultural challenges.

2. Materials and Methods

2.1. Genetic Materials

The genetic material used in this study included twelve pigmented rice genotypes from various regions in Indonesia (Table 1). The diversity of sampling locations provides an opportunity to analyze genotype variation, aiming to support pre-breeding programs by identifying unique traits of pigmented rice genotypes from different regions.

Genotypes were selected to represent pigmented and non-pigmented rice landraces from regions known for the traditional cultivation of local varieties, including Majalengka (West Java), Toraja (South Sulawesi), Kutai Kartanegara (East Kalimantan), and Yogyakarta. Selection was based on phenotypic diversity, particularly grain pericarp color, and the cultural relevance of the varieties. In Toraja, several genotypes such as Pare Bau, Pare Lea, Pare Ambo, and Pare Lontong Tanduk have been cultivated for generations and are well-documented for their genetic richness (Zulkifli Maulana 2017). Although molecular documentation is limited in the other regions, local farmers are known to preserve traditional varieties with unique traits, suggesting unexplored genetic potential. Genotypes from BRMP Biogen and BB Padi were included as national reference varieties.

2.2. Experimental Design

The experimental design employed in this study was a completely randomized design (CRD) with three replications. Each experimental unit comprised one

Table 1. Materials used in this research

Materials	Pigment	Place of origin
Maja	Black	Majalengka, West Java
Cicurug Maja	Black	Majalengka, West Java
Jeliteng	Black	Sleman, Special Region of Yogyakarta
Pare Ambo	Black	Toraja, South Sulawesi
Pare Lontong Tanduk	Black	Toraja, South Sulawesi
Habang Separi	Red	Kutai Kartanegara, East Kalimantan
Maja Kulur	Red	Majalengka, West Java
Pare Lea	Red	Toraja, South Sulawesi
Pare Bau	White	Toraja, South Sulawesi
Bioryza Agritan	White	BRMP Biogen
Biomonas Agritan	White	BRMP Biogen
Ciherang	White	BB Padi

clump of plants. The seeds were first germinated for 14 days before being transferred to buckets. Each genotype was planted in three pots, with two seedlings per pot. Replanting was carried out to replace dead plants as soon as they were identified, up to two weeks after planting. Watering was performed every two days to maintain soil moisture. Control measures for weeds, pests, and diseases were implemented as needed based on field conditions and applied when infestations occurred.

2.3. Observation

The agronomic traits observed included quantitative and qualitative traits. The quantitative traits were plant height (cm), total number of tillers and productive tillers (number of panicles), flowering time, maturing time, panicle length (cm), number of filled grains, number of unfilled grains, total number of grains per panicle, percentage of filled grains (%), percentage of unfilled grains (%), 100-grain weight (g), seed length, seed width, and seed length-to-width ratio. Quantitative traits were recorded based on Bioversity International 2007 and International Rice Research Institute 2013. The qualitative traits observed included the presence of awns and secondary panicle branching (recorded based on Bioversity International 2007), awn color, and pericarp color, as determined by the RHS color chart.

2.4. DNA Isolation and PCR Conditions

Total genomic DNA from all genetic materials used in this study was isolated from the leaves using the CTAB method (Doyle and Doyle 1990). DNA concentration was estimated using a NanoDropTM2000 (Thermo Scientific, USA), while DNA purity was assessed based on absorbance ratio values of A_{260}/A_{280} and A_{260}/A_{230}. The PCR reaction was carried out in a total volume of 10 µL, consisting of 2.5 µL of 20 ng μL^{-1} genomic DNA, 2.5 µL of primers at a concentration of 10 mM, and 5 µL of 2x MyTaq^{TM} HS Red Mix. The primers used for analyzing pigmented rice genetic diversity are listed in Supplementary 1 Table 1. The PCR program included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles consisting of denaturation at 94°C for 5 seconds, annealing at 50°C for 60 seconds, and elongation at 72°C for 30 seconds. The PCR reaction concluded with a final elongation step at 72°C for 10 minutes. The amplicons from the SCoT markers were analyzed by electrophoresis on 1.5% (w/v) agarose gel at 90 V for 90 minutes in 0.5× TBE buffer. The gel was stained in 0.5 µg mL^{-1} Ethidium Bromide solution for 15 seconds and rinsed with distilled water for 5 minutes before being visualized using a UV transilluminator.

2.5. Data Analysis

All quantitative trait data were analyzed using analysis of variance (ANOVA) with F-tests. If significant differences were observed, further analysis was conducted using the Honest Significant Difference (HSD) test at a 5% level of probability using Minitab software. Genetic analysis was carried out by estimating broad-sense heritability (h^2bs) and the genetic coefficient of variation. Broad-sense heritability was calculated based on the variance components derived from the mean performance of genotypes across replicated trials, following the method described by Sobir and Syukur (2015) and Burton and DeVane (1953). Categories for Genetic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) were based on Deshmukh *et al.* 1986, with low values ranging from 0-10%, moderate values from >10-20%, and high values above >20%. Heritability was categorized as high if $h^2bs > 60\%$, moderate if $30\% \leq h2bs \leq 60\%$, and low if $h^2bs < 30\%$, following Burton and DeVane 1953. Principal Component Analysis (PCA) was conducted using R Studio.

Molecular data analysis was conducted by scoring DNA bands from visualized DNA. Band scoring was performed using GelAnalyzer software. The scoring data were analyzed using iMEC (Online Marker Efficiency Calculator) (Amiryousefi *et al.* 2018) to determine the expected polymorphic information content (PIC). All the quantitative, qualitative, and molecular data were then analyzed using the Gower Dissimilarity Method in PBSTATCL (www.pbstat.com), and the results were presented in the form of a dendrogram.

3. Results

3.1. Agronomic Performance

The agronomic and yield performance of pigmented rice genotypes are summarized in Table 2 and Supplementary 2 Table 2. Among black rice genotypes, Pare Ambo exhibited the tallest plant height (180 cm) and the longest panicle length (31.61 cm), suggesting its potential for high grain production. In contrast, Cicurung Maja had the shortest panicle length (23.22 cm). The red rice genotype Habang Separi had the highest number of filled grains per panicle (138.67), while Pare Lontong Tanduk had the fewest unfilled

Table 2. Agronomic traits performance of pigmented rice

Genotype	Pigment	Plant height	Number of tillers	Productive tillers	Days to heading	Days to maturity
Maja	Black	115.67^{d}	8.00^{de}	7.33^{bcd}	141.00^{c}	182.67^{c}
Cicurug Maja	Black	111.83^{def}	8.00^{de}	7.33^{bcd}	143.33^{c}	179.33^{cde}
Jeliteng	Black	105.25^{def}	12.00^{ab}	10.00^{ab}	108.00^{d}	170.00^{cdef}
Pare Ambo	Black	180.00^{a}	9.33^{bcd}	9.33^{abc}	143.33^{c}	188.33^{c}
Pare Lontong Tanduk	Black	170.00^{ab}	9.00^{cd}	9.00^{abcd}	145.00^{c}	176.00^{cde}
Habang Separi	Red	113.67^{de}	12.33^{a}	10.33^{ab}	147.33^{c}	182.00^{c}
Maja Kulur	Red	109.33^{def}	9.67^{abcd}	9.67^{abc}	139.00^{c}	180.33^{cd}
Pare Lea	Red	152.67^{bc}	11.67^{abc}	11.67^{a}	175.33^{b}	214.33^{b}
Pare Bau	White	141.67^{c}	8.00^{de}	8.00^{bcd}	218.00^{a}	257.67^{a}
Bioryza	White	94.33^{f}	7.67^{de}	6.33^{cd}	107.67^{d}	159.67^{ef}
Biomonas	White	97.00^{ef}	5.67^{e}	5.67^{d}	106.67^{d}	151.67^{f}
Ciherang	White	103.33^{def}	8.67^{d}	7.67^{bcd}	125.33^{c}	161.67^{def}

grains. The white rice genotype Pare Bau displayed the longest maturity period (257.67 days), significantly different from others based on the HSD test.

PCA results (Figure 1) revealed distinct clustering of genotypes based on their traits, with Pare Ambo and Pare Lontong Tanduk grouped in the first quadrant due to moderate numbers of tillers and productive tillers, while Habang Separi, Pare Lea, and Pare Bau clustered in the second quadrant, characterized by early flowering and a high number of filled grains. Genotypes such as Maja Kulur, Cicurug Maja, and Bioryza grouped in the third quadrant were associated with traits such as seed length and grains per panicle. In contrast, Jeliteng, Ciherang, and Biomonas in the fourth quadrant were influenced by traits such as a high number of grains.

Qualitative traits such as awn presence and aleurone color varied across genotypes (Table 3, Figures 2 and 3). Genotypes like Maja, Jeliteng, and Biomonas lacked awns, while Pare Bau displayed awns along the entire panicle. Aleurone colors ranged from black in black rice genotypes to reddish brown and white in red and white rice genotypes, respectively. These findings, supported by PCA, highlight significant variations in morphological, agronomic, and yield traits, emphasizing the potential of specific genotypes for breeding and cultivation programs.

3.2. Variance Components, Heritability, Genetic Coefficient of Variation, and Phenotypic Coefficient of Variation in Pigmented Rice

The variance components, heritability, and genetic and phenotypic coefficients of variation (GCV and PCV) were analyzed for several agronomic traits in pigmented rice (Table 4). The GCV ranged from 4.42 to 23.18%, with most traits categorized as moderate to high, except for traits such as seed length, percentage of filled grains, and panicle length, which exhibited low GCV values (<10%). Across all traits, the PCV consistently exceeded the GCV, highlighting the significant environmental influence on these traits. Broad-sense heritability estimates varied from 37 to 98%, with traits such as plant height, number of tillers, productive tillers, flowering time, maturing time, panicle length, seed traits, and length-to-width ratio demonstrating high heritability (>50%). In contrast, traits such as the number of unfilled grains, percentage of unfilled grains, and grains per panicle displayed moderate heritability (20–50%). These findings suggest that selection for highly heritable traits may effectively improve genetic gain, whereas traits with moderate heritability may require further evaluation under diverse environmental conditions.

3.3. Polymorphism Analysis

In this study, SCoT marker polymorphism was calculated and measured (Table 5). A total of 229 amplicons were successfully amplified using 23 SCoT markers. SCoT marker 21 produced the highest number of amplicons (17), while SCoT marker 8 yielded the lowest number (2). The polymorphism percentage ranged from 13% (SCoT primer 23) to 92% (SCoT primer 14). The average polymorphism percentage was 68%, reflecting substantial genetic variation among the genotypes analyzed. The Polymorphism Information Content (PIC) values for all primers ranged from 0.3610 to 0.4646, with an average value of 0.3902. Most primers exhibited high PIC values (>0.30), indicating that the SCoT markers used were highly informative in detecting genetic diversity among the pigmented rice genotypes. High PIC values (0.3610–0.4646) further confirm the markers' informativeness.

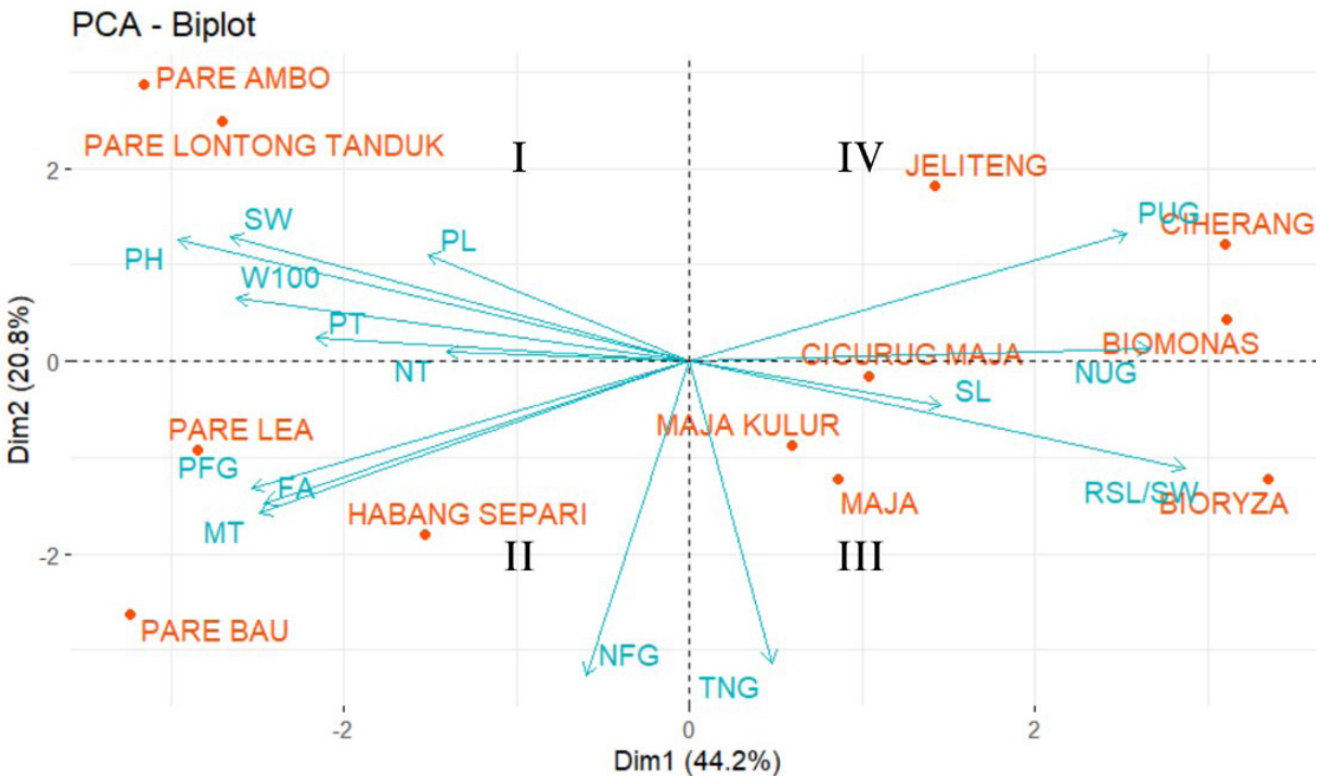

Figure 1. Principal component analysis (PCA) of pigmented rice based on quantitative traits

Table 3. Qualitative traits performance of pigmented rice

Genotype	Awns	Awns color	PSB	AC
Maja	None	Absent	Sparse	Black
Cicurug Maja	None	Absent	Sparse	Black
Jeliteng	None	Absent	Absent	Black
Pare Ambo	Tip Only	Black	Clustered	Black
Pare Lontong Tanduk	Whole length	Black	Clustered	Black
Habang Separi	None	Absent	Sparse	Reddish brown
Maja Kulur	Tip Only	Straw	Sparse	Reddish brown
Pare Lea	None	Absent	Absent	Reddish brown
Pare Bau	Whole length	Straw	Sparse	White
Bioryza	None	Absent	Absent	White
Biomonas	None	Absent	Absent	White
Ciherang	None	Absent	Absent	White

3.4. Clustering of Genetic Diversity in Pigmented Rice using Gower's Method

The cluster analysis based on the Gower coefficient revealed four distinct groups of pigmented rice genotypes, as depicted in the dendrogram (Figure 4). The Gower coefficient was chosen for its ability to handle mixed data types, which in this study included agronomic traits and molecular marker-based SCoT analysis. Data standardization was performed prior to the calculation of Gower distances to ensure valid comparisons among the 12 rice genotypes.

The results showed that the first group consisted of the genotypes Biomonas and Bioryza, indicating a high degree of genetic similarity. The second group included the genotypes Maja Kulur and Cicurug Maja. The third group clustered the genotypes Jeliteng, Maja, and Habang Separi. In contrast, the fourth group comprised the genotypes Pare Lontong Tanduk, Pare Lea, Pare Ambo, Pare Bau, and Ciherang. This grouping demonstrates substantial genetic variation among the studied pigmented rice genotypes. This clustering reflects the genetic differentiation associated with rice

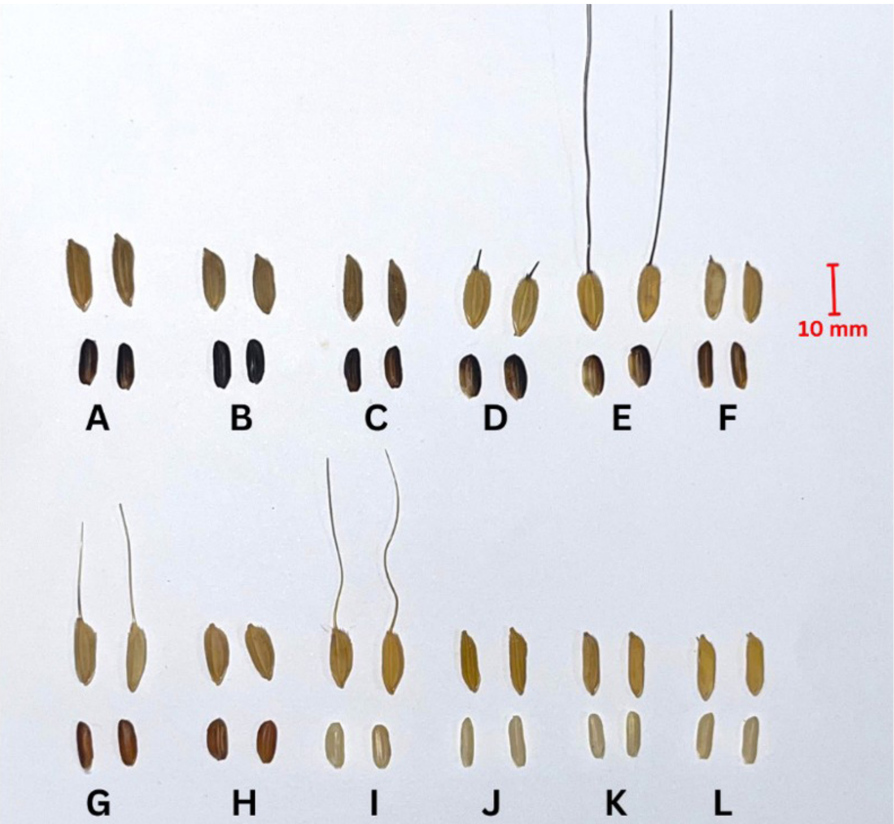

Figure 2. Phenotypic difference of grain color across 12 Indonesian pigmented rice genotypes. (A) Maja, (B) Cicurug Maja, (C) Jeliteng, (D) Pare Ambo, (E) Pare Lontong Tanduk, (F) Habang Separi, (G) Kulur, (H) Pare Lea, (I) Pare Bau, (J) Bioryza, (K) Biomonas, (L Ciherang.

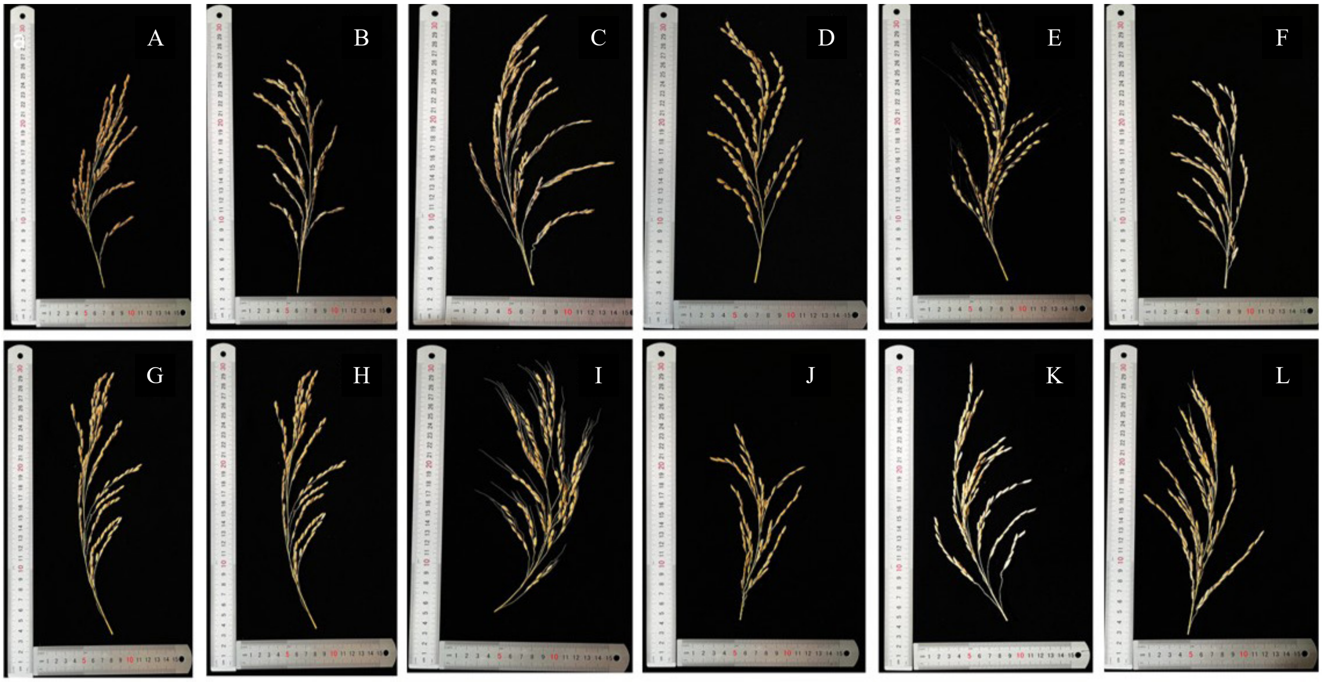

Figure 3. Phenotypic difference of panicle across 12 Indonesian pigmented rice genotypes. (A) Maja, (B) Cicurug Maja, (C) Jeliteng, (D) Pare Ambo, (E) Pare Lontong Tanduk, (F) Habang Separi, (G) Kulur, (H) Pare Lea, (I) Pare Bau, (J) Bioryza, (K) Biomonas, (L) Ciherang

Table 4. Summary of analysis of variance components, heritability, genetic coefficient of variation, and phenotypic coefficient of variation in pigmented rice

Trait	MSG	CV(%)	σ^2e	σ^2g	h^2bs (%)	GCV(%)	PCV(%)
PH	2538.88	4.90	37.25	833.88	833.88	23.18	23.69
NT	11.85	9.91	0.83	3.67	3.67	20.91	23.15
PT	9.36	13.60	1.35	2.67	2.67	19.16	23.51
FT	2867.46	3.12	19.91	949.18	949.18	21.56	21.78
MT	2400.39	3.62	44.16	785.41	785.41	15.26	15.68
PL	20.92	5.63	2.19	6.24	6.24	9.51	11.05
NFG	1510.73	18.51	452.15	352.86	352.86	16.35	24.70
NUG	0.63	39.45	0.23	0.13	0.13	14.58	24.07
TNG	1585.63	16.60	550.46	345.06	345.06	13.14	21.17
PFG	125.39	8.16	43.97	27.14	27.14	6.41	10.38
PUG	0.41	35.34	0.13	0.09	0.09	14.43	22.32
W100	0.31	3.96	0.01	0.10	0.10	13.02	13.65
SL	0.50	3.49	0.09	0.14	0.14	4.42	5.70
SW	0.38	6.44	0.03	0.12	0.12	12.97	14.54
RSL/SW	0.67	7.05	0.05	0.21	0.21	14.05	15.66

MSG: mean square genotype, CV: coefficient of variation, PH: plant height, NT: number of tillers, PT: productive tillers, FA: flowering time, MT: maturing time, PL: panicle length, NFG: number of filled grains, NUG: number of unfilled grains, TNG: total number of grains per panicle, PFG: percentage of filled grains, PUG: percentage of unfilled grains, W100: weight of 100 filled grains, SL: seed length, SW: seed width, RSL/W: seed length-to-width ratio

Table 5. Polymorphism analysis results of SCoT primers amplification in pigmented rice

Primer name	Total number of bands	Number of polymorphism bands	Polymorphic band (%)	Polymorphism information content
SCoT 3	11	8	73	0.3829
SCoT 7	13	8	62	0.4403
SCoT 8	2	1	50	0.4646
SCoT 11	12	6	50	0.4568
SCoT 12	13	7	54	0.4270
SCoT 13	14	11	79	0.3621
SCoT 14	12	11	92	0.3672
SCoT 20	8	7	88	0.3965
SCoT 21	17	15	88	0.3750
SCoT 22	13	11	85	0.3610
SCoT 23	15	2	13	0.3866
SCoT 25	12	10	83	0.3866
SCoT 26	10	8	80	0.3842
SCoT 27	4	3	75	0.3621
SCoT 28	11	10	91	0.3714
SCoT 29	7	5	71	0.3716
SCoT 30	11	4	36	0.3661
SCoT 31	7	6	86	0.3980
SCot 32	5	4	80	0.3775
SCoT 33	4	2	50	0.4151
SCoT 34	9	7	78	0.3612
SCoT 35	9	7	78	0.3984
SCoT 36	10	2	20	0.3621
Mean	10	7	68	0.3902

subspecies and phenotypic traits, which is valuable for breeding selection.

4. Discussion

The results of this study provide essential insights for the development of superior pigmented rice varieties. The identification of genotypes with high agronomic performance, combined with traits exhibiting high heritability, offers valuable opportunities for direct selection in breeding programs. The presence of substantial genetic variability, as revealed by morphological and molecular analyses, supports the strategic selection of diverse parental

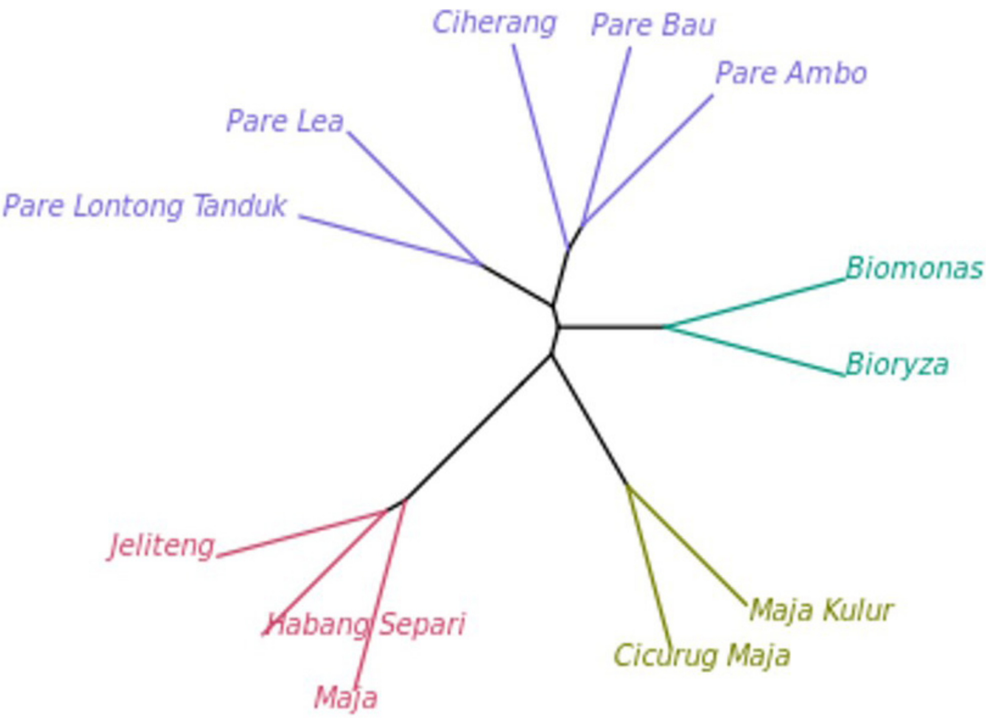

Figure 4. Cluster analysis of 12 pigmented rice genotypes based on grouping analysis using the Gower dissimilarity method

lines to broaden the genetic base and enhance hybrid performance. Genotypes grouped into different clusters based on agronomic and molecular data can serve as candidates for targeted crossing to produce new lines with improved yield potential, resilience, and desirable grain qualities. Furthermore, the informative nature of the SCoT markers used in this study demonstrates their potential utility in marker-assisted selection to accelerate the development of improved cultivars. These findings collectively highlight the applicability of the research in supporting pre-breeding activities and guiding the systematic improvement of pigmented rice germplasm.

4.1. Phenotypic Characteristics

Understanding key agronomic traits is essential for improving rice productivity and adaptability. Among these traits, plant height significantly influences yield components, with taller genotypes prone to lodging and shorter ones susceptible to submergence in flood-prone areas, both leading to yield loss. Therefore, breeding efforts aim to produce moderately tall plants (Khan *et al.* 2015). The number of tillers contributes to grain yield; however, Chowhan *et al.* (2017) observed that higher tiller counts could reduce grain filling efficiency, decreasing yield and quality (Nortona *et al.* 2017). Flowering time, marked by panicle emergence, and maturing time, recorded at physiological grain maturity, are critical traits that determine planting indices within a year (Prayoga *et al.* 2018). QTLs/genes and environmental factors influence variations in these traits (Demeke *et al.* 2023).

Genetic variability indicators, including the genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2bs), and genetic advance, aid breeders in selecting suitable genotypes for hybridization (Hossain *et al.* 2015). GCV values are categorized as low (0–10%), moderate (>10–20%), and high (>20%) (Burton and Devane 1953). Low GCV indicates limited genetic variability (Belay *et al.* 2024). Heritability is considered high when $h^2bs > 60\%$, moderate when $30\% \leq h^2bs \leq 60\%$, and low when $h^2bs < 30\%$ (Burton and Devane 1953). High heritability, as reported by Demeke *et al.* 2023, was observed for traits such as plant height, tiller number, flowering time, maturing time, and panicle length, making them reliable selection criteria for breeding (Terfa and Gurmu 2020). High heritability traits accelerate the identification and improvement of desirable genetic traits in breeding programs (Jahan *et al.* 2020).

The relationship between rice yield and its components has been extensively studied at the phenotypic level (Akinwale *et al.* 2011). Principal component analysis (PCA) identifies traits contributing to genetic diversity and characterizes genotypes

(Afuape *et al.* 2011). The proximity of genotypes in the PCA biplot reflects a high degree of similarity in traits (Kassambra and Mundt 2020). These findings provide insights for enhancing breeding strategies and optimizing trait selection for improved rice varieties.

4.2. Genetic Characteristics

Primer selection is a crucial step in genetic diversity analysis using SCoT markers, as it affects the accuracy, reproducibility, and informativeness of the results (Omar *et al.* 2023). In this study, SCoT primers demonstrated good performance in detecting genetic diversity. SCoT primer 21, which produced the highest number of amplicons (17), showed optimal amplification capacity, whereas SCoT primer 8, with only 2 amplicons, indicated limitations in detecting polymorphism. The polymorphism percentage of 92% observed with SCoT primer 14 highlights its effectiveness in detecting genetic variation. In contrast, the lowest polymorphism percentage (13%) was found with SCoT primer 23 (Figure 5), reflecting its limited ability to reveal variation among genotypes.

The PIC values in this study ranged from 0.3610 to 0.4646, classified as high according to Serrote *et al.* 2020 This demonstrates the strong capability of SCoT markers in detecting genetic diversity. These findings suggest that SCoT markers can be widely applied to both pigmented and non-pigmented rice genotypes. With their ability to detect significant genetic variation, these markers hold great potential for use in rice breeding programs to enhance genetic diversity and productivity (Serrote *et al.* 2020).

4.3. Genetic Diversity Clustering in Pigmented Rice using Gower's Method

The clustering of genotypes into four distinct groups highlights significant genetic variation based on agronomic traits and molecular data. The use of the Gower coefficient allowed for a comprehensive analysis by integrating mixed data types, as described by Romesburg 1984. The standardization of data prior to analysis ensured that differences in measurement scales did not affect the results. The first group, comprising Biomonas and Bioryza, reflects a close genetic relationship, potentially indicating adaptation to similar environments or shared genetic backgrounds. The second group (Maja Kulur and Cicurug Maja) also displayed strong genetic similarity, which could be further explored to identify specific advantageous traits. The third group (Jeliteng, Maja, and Habang Separi) exhibited unique genetic characteristics compared to the other groups, potentially contributing valuable traits for breeding programs. The fourth group, consisting of Pare Lontong Tanduk, Pare Lea, Pare Ambo, Pare Bau, and Ciherang, showed broader genetic diversity, offering greater opportunities for selecting genotypes

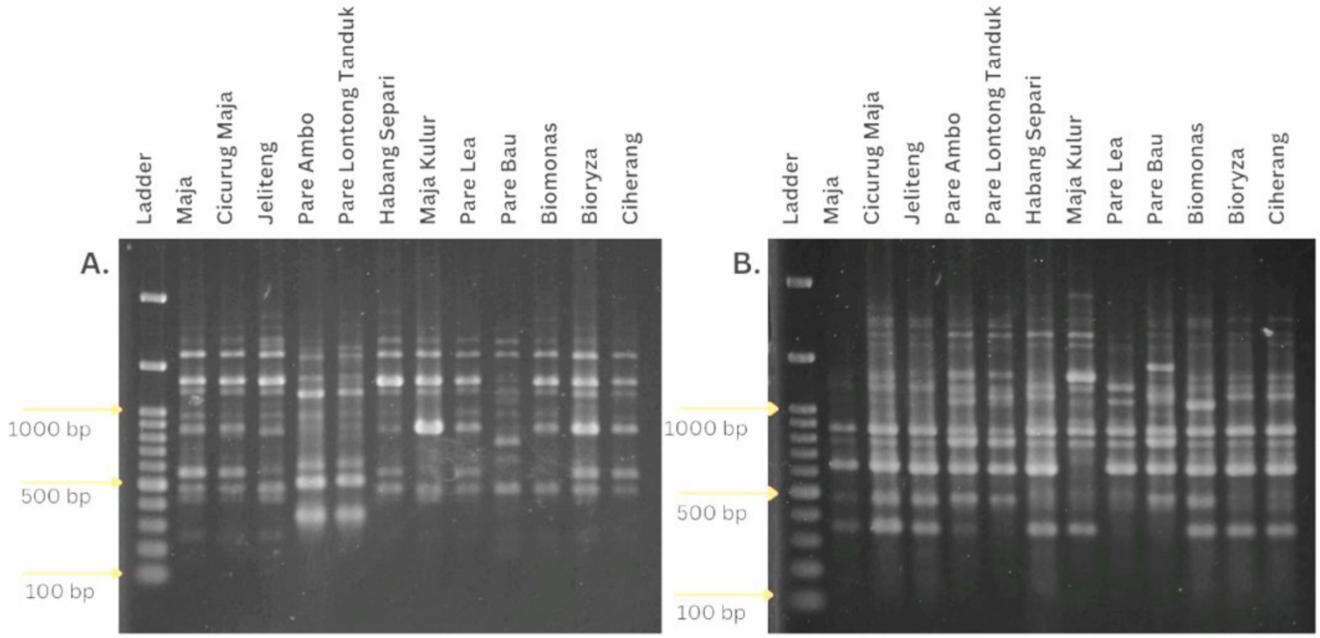

Figure 5. (A) Visualization of amplicon band amplified by SCoT 7 marker across 12 Indonesian pigmented rice genotypes, (B) Visualization of amplicon band amplified by the SCoT 23 marker across 12 Indonesian pigmented rice genotypes

with superior traits. These findings emphasize the importance of understanding genetic diversity to support breeding programs. The identified genetic variation can be leveraged to enhance agronomic traits, improve productivity, and increase adaptability to specific environmental conditions.

The integration of PCA and Gower dissimilarity analysis offers a robust approach to understanding genetic diversity among pigmented rice genotypes by utilizing both quantitative and qualitative traits. PCA highlights traits with significant contributions to genetic variance, such as yield and agronomic traits, while Gower analysis complements it by incorporating both quantitative and qualitative data to calculate genetic distance and group genotypes with similar characteristics (Hinton and Salakhutdinov 2006; Klein *et al.* 2024). The observed clusters, such as the grouping of Jeliteng, Habang Separi, and Maja based on shared traits like plant height and the absence of awns, demonstrate the interplay of morphological traits. Similarly, the clustering of genotypes from Tana Toraja, such as Pare Bau and Pare Ambo, reflects the influence of both regional origin and trait similarity.

These findings underscore the potential of Gower's analysis to capture genetic relationships comprehensively, integrating multiple data types to inform breeding strategies. The high genetic diversity observed is crucial for strengthening breeding programs aimed at developing pigmented rice varieties with enhanced productivity and resilience to diverse environmental conditions (Sofian *et al.* 2019; Al-Khayri and Jain 2024). The integration of genetic diversity analysis into plant breeding efforts ensures the sustainable development of superior rice varieties, aligning with long-term goals for agricultural improvement and food security.

This study underscores the importance of genetic diversity analysis in pigmented rice for improving agronomic traits, productivity, and adaptability. The effectiveness of SCoT primers in detecting genetic variation, combined with the robust clustering approach using the Gower method, provides valuable insights for breeding programs. The integration of morphological and molecular data revealed significant genetic variation and highlighted key genotypes for further exploration.

Based on the results of this study, it's advisable to incorporate selected genotypes exhibiting superior agronomic performance and high genetic variability into breeding programs as parental lines to improve yield potential and grain quality in pigmented rice. The utilization of SCoT markers may be further optimized to support early-generation selection, particularly for traits with high heritability, thereby enhancing the effectiveness and precision of selection strategies. Future studies should include multilocation field trials to validate the stability of agronomic traits across diverse environmental conditions. Furthermore, advanced molecular approaches such as functional gene expression analysis and genome-wide association studies are recommended to identify candidate genes associated with pigmentation, yield-related traits, and abiotic stress tolerance. The integration of molecular, physiological, and genomic data is expected to facilitate the development of high-performing and climate-resilient pigmented rice cultivars, contributing to long-term goals in crop improvement and food security.

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