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Application of SNP Markers to Identify Genetic Variation on Mutant Lines of Adan-Krayan, Special Local Rice from North Kalimantan

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Abstract—Adan rice, a local rice from the Krayan highland of North Kalimantan is marked by its fine taste that consumers like. However, this rice can only be planted once a year due to its long lifespan. Efforts have been made to develop a short-lived version of Adan, to enable it to be planted twice a year. Adan Kelabit was initially exposed to gamma radiation, which produced several mutants. Field selection identified eight mutants with shorter lifespans than the Adan. In this study, these eight mutants were observed further. SNP markers were carried out to confirm the occurrence of mutations, while observations of agronomic characters and grain quality were carried out to determine the most promising lines. The molecular study showed mutations found in all chromosomes of the mutants and differentiated them from the original Adan, with the similarity level ranging from 0.746 to 0.843. However, this closeness is not correlated to the dosage of the gamma rays applied. The majority of the mutants have shorter plant height and higher yield potential. On average, the mutant is 11.5% shorter on heading date, 22.69% higher on tiller number, 27.14% higher on grain weight, and 26.64% higher on other parameters of yield potential. Apart from the shorter heading date, each mutant has its superiorities. The A25 and A28 mutant lines have much higher yield potential while maintaining the same level of amylose content as the Adan rice. These two mutants can be considered for further development or dissemination for farmer adoption.

Keywords—Adan rice; mutation; genetic diversity; SNP markers; agronomic characters; rice quality.

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I. INTRODUCTION

Adan rice, a local rice from Krayan district, Nunukan Regency, North Kalimantan Province, Indonesia, is considered indigenous premium rice of this province. It is known for its small, elongated grains, white-like crystals, aromatic, fluffier texture, and delicious taste. This rice is a legacy of the ancestors, and it has been organically cultivated and passed down for generations by a local community of the Dayak tribe. Several varieties are cultivated in Krayan, including Adan Lumbis, Adan Merah, Adan Kelabit, Adan Putih, Adan Hitam, and Adan Saleh. This organic, highly nutritious rice is a dietary staple for officials and Brunei Darussalam residents and is often served to the royal family. Located in a peripheral area, the Krayan area is relatively isolated from neighboring districts in Indonesia. This area is more accessible to Malaysia than to Indonesian consumers. Thus the price is also more affordable for the Malaysian market [1].

Adan rice was registered for GI in 2012. Adan rice is currently safeguarded by Geographic Indication (GI) status, having received official protection from the government through the Ministry of Law and Human Rights via Geographical Indication Certificate: IG.00.2011.000004 ID G 000000013, issued on January 6, 2012 [2]. In Malaysia, the Kelabit community cultivated Adan rice in the highlands of Northern Sarawak, which is locally known as Bario rice. The rice has also been registered for GI with the Malaysian Intellectual Property Organization (https://www.fondazioneslowfood.-com/en/ark-of-taste-slow-food/bario-rice/).

The development of Adan rice needs to be improved by several factors, especially its long maturity. Because of this long maturity, rice plantation can be conducted only once a year. This cultivation system could be more economical. Heading date in rice is controlled by multiple factors, including complex epigenetic regulation and integration mechanism between photoperiod and circadian clock [3]. Therefore, efforts were made to improve Adan rice using gamma-ray radiation to shorten its growth duration. The use of mutations as an alternative way to enhance genetic diversity is also commonly used in breeding programs [4]. For instance, to increase salinity and drought tolerance [5] and blast resistance [6].

Gamma rays have been used in mutagenesis to enhance the growth duration and productivity of Adan rice in Indonesia. The Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD) and the Local Government of East Kalimantan Province have collaborated on shortening Adan rice's maturity through gamma-ray mutations since 2011, studying mutants for molecular and agronomic aspects. Its genetics, the traditional cultivation system [1], and its natural environment probably determines its unique taste. A study of several Adan rice showed high nutritional values such as protein, iron content, and vitamin [7].

Along with applying mutation and biotechnological approaches, DNA markers are generally used to accelerate the breeding program. The combination of modern techniques and the high throughput screening method assisted by DNA markers become critical roles in the success of crop development. Single nucleotide polymorphism (SNP) markers are one of the molecular markers recently developed rapidly and simultaneously with advances in genome sequencing technology using next-generation sequencing (NGS). SNP markers show one base at a specific location between rice accessions which can then be used for various kinds of genetic analysis [8], [9]. Morales et al. [10] reported that an SNP array containing 7,098 markers had been developed from the Cornell-IR LD Rice Array (C7AIR). In addition, Rice3K56 containing 56,606 SNP markers [11] and 580K SNP array [12] had also been developed. SNP markers for selecting essential traits in rice are also starting to be assembled [13], [14], [15].

The Indonesian National Standard (SNI) classifies rice based on criteria such as milling degree, moisture content, and grain quality attributes, reflecting the shift in rice breeding towards prioritizing quality alongside yield There are two quality classes of rice: premium and medium, and there is also specialty rice for specific purposes, such as rice for diabetics or glutinous rice. The nutritional composition of Indonesian specialty cereals, including rice, corn, and sorghum, is also of interest, as these local varieties can provide essential nutrients and potentially combat malnutrition [16].

The general requirements include the free condition of the material from pests and diseases, undesirable odors, and dangerous chemicals, whereas the specific requirements include milling degree, moisture content, head rice, broken rice, brewers rice, red rice, yellowish grain, chalky grain, and undesired strange materials [17]. Specific functional characteristics such as amylose and protein content specifically increase the preference in some consumers. This is in line with the development of rice breeding, which began to precipitate quality, in addition to yield [18]. Gong et al. [19] reported that several researchers had created QTL markers related to grain quality, such as QTLs associated with critical traits, including appearance, aroma, texture, and nutritional properties. Therefore, selected Adan rice mutants need to be detected using SNP markers so that mutations can be confirmed. Agronomic characters and grain quality must also be further studied in Adan rice mutants.

II. Materials and Method

This study continues the serial steps of the rice breeding program conducted from 2010 to 2016. The study utilized M_5 mutant lines selected in the field (Table I), along with Adan Kelabit rice, a subtype of white Adan rice. Gamma-ray radiation treatment, ranging from 0 to 250 Gray (Gy), was administered at the onset of material formation at the Center for Isotope and Radiation Application, National Atomic Power Agency, Jakarta. Several mutants were produced from the mutation program of Adan Kelabit rice through a gamma-radiation approach carried out at the Center for Isotope and Radiation Application of the National Atomic Power Agency. Eight individuals of fifth-generation mutants (M_5) were targeted for this study. These mutants were selected from 115 mutants. These mutants have similar performance (shape) to the parent and have early maturity character (Fig. 1).

A. SNP Analysis

The SNP analysis was conducted at the International Rice Research Institute (IRRI), Philippines. A 6K SNP chip Cornell_6K_Array_Infinium_Rice (C6AIR) consisting of 6,000 SNP markers [20] was used. Genomic DNA extraction followed the method outlined by Dellaporta et al. [21], isolating DNA from both young and fresh leaves of eight mutants of Adan rice and their parent. The isolated DNA was promptly desiccated and sealed with parafilm in microtubes before transportation to IRRI for further processing. Data, comprising DNA bases (AA, AG, AC, etc.), were analyzed using Flapjack software (https://ics.hutton.ac.uk/flapjack/download-flapjack/), encompassing SNP profiles, a similarity matrix, and dendrogram generation. The similarity matrix was generated using the R dist() command, while dendrogram construction utilized the R hclust() method.

TABLE I
MUTANTS OF ADAN RICE SELECTED IN THE FIELD

No	Generation	Lines	Radiation dose (Gy)
1	-	Adan	0
2	M_5	A25	100
3	M_5	A27	100
4	M_5	A28	100
5	M_5	A50	150
6	M_5	A51	150
7	M_5	A88	150
8	M_5	A93.1	150
9	M_5	A93.2	150

B. Agronomic Analysis

The agronomic assessment of the Adan rice mutants took place at Muara Field Station in Bogor, spanning from April to

August 2016. The experimental site features Latosol soil at 240 m above sea level. Each mutant was cultivated in sizable plots measuring 10 m by 13 m, with a planting spacing of 25 cm by 25 cm. Employing an organic approach, the planting procedure encompassed fertilizer application, treatment methods, and pesticide use. Seeds utilized were harvested at 21 days after sowing (DAS).

Seed 21 DAS were planted and maintained in an organic system. Field observations were conducted for heading date, plant height, and productive tiller number. Post-harvest observations were performed on panicle length, grain number per panicle (empty grain, filled grain, and the total), grain weight per plot, per plant, and the 100-grain weight at 14% moisture content. A simple statistical analysis was performed to assess the agronomical performance.

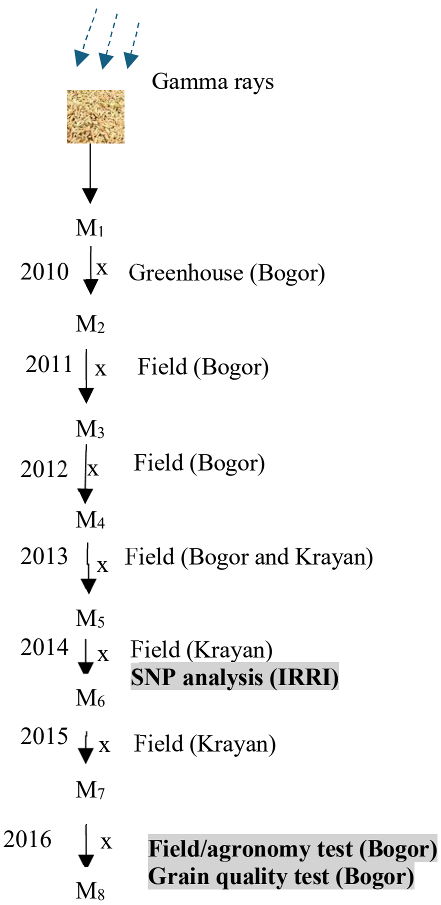


Fig. 1 Schematic of the mutation and selection process of Adan Krayan rice. x = self-crossing. The bold and shaded parts are the material for this publication.

C. *Rice Grain Quality Test*

Two kilograms of milled grain of each sample were tested for its physical and chemical/cooking qualities. The grain quality testing was conducted at the laboratory service of the Indonesian Center for Post-Harvest Research Institute, The Indonesian Agency for Agricultural Research and Development (IAARD). The characteristics of the physical quality include milling characteristics, whiteness, and chalkiness, while the chemical characteristics include gelatinization temperature, amylose content, gel consistency, and viscosity. Nutritional content was also observed for lipids and proteins. The physical grain quality tests were performed according to the Indonesian National Standard (SNI 6128-2008), whereas the chemical quality tests were performed according to the method of Soxhlet, Kjeldahl, and spectrophotometry for the test of lipid, protein, and amylose content, respectively.

III. Results and Discussion

The Mutation approach is commonly used in breeding programs. Among various techniques of mutation breeding, physical radiation is more widely applied. In this technique, the radiation is applied to induce DNA damage and induce. Particular mutations such as deletion, insertion, inversion, and translocation can lead to phenotypic change [22]. Thus, mutation is widely used to increase crop performance. This approach has been used for nearly 100 years [23]. In Indonesia, a total of 28 improved varieties were developed through physical mutation, which is the highest among Southeast Asia countries [24].

This research endeavor successfully identified early-maturing mutant lines of Adan rice. While conducting selection activities in the origin region, namely the Krayan district, proves highly beneficial, logistical challenges arise due to its remote and inaccessible location, often necessitating alternative selection and planting sites. Consequently, agronomic and rice quality data collection may only sometimes occur in the area. From the mutant selection spanning 2011 to 2014, specific mutants demonstrated viability, maturing even after radiation doses of 100 and 150 Gy. In contrast, doses of 200 and 250 Gy seeds needed to be attainable (Table I). The parent, Adan Kelabit rice, represents a prevalent variety among farmers in Krayan. At a certain dosage, gamma-ray-induced changes in rice typically lead to a reduction in germination [25]). In this instance, doses of 200 and 250 Gy did not result in any plant growth at Adan rice.

A. *SNP Analysis*

According to the I Scan tool analysis, out of the 6,000 SNP markers utilized in this study, 4,606 markers were successfully read, while 1,394 markers (23.23%) could not be read. This discrepancy may arise due to the random distribution of SNPs across rice chromosomes, resulting in markers incompatible with the study's samples.

Most detected alleles appeared in homozygous conditions, predominantly GG, CC, AA, and TT, with a smaller proportion being --, AG, TC, AC, and TG. Notably, alleles such as AT, TA, CA, CT, CG, GA, GT, and GA were undetected (data needs to be displayed). Conversely, heterozygous allele patterns were still present in the Adan rice parents, albeit at a low frequency of approximately 2.13%. It highlights the inevitability of homozygous allele variations in plants considered phenotypically homogeneous in the field, with allele deviation remaining below 5%.

Using SNP markers to detect Adan rice mutants has demonstrated remarkable efficacy. Analysis revealed successful examination of 4,606 SNP markers, averaging approximately 383.83 markers per chromosome, thus indicating a notably dense distribution across each chromosome. In contrast, previous studies such as Prasetiyono et al. [26] employed fewer SSR markers, with only 71 markers utilized to assess genetic variations among eight selected Adan rice mutants. However, in the current study, a significantly more significant number of SNP markers, totaling 4,606, were employed, representing an increase of 64.87 times the number of SSR markers typically used in Adan rice mutation research.

To discern allele variations between mutants and Adan rice, one can examine the genetic relationships among mutants via the similarity matrix and the dendrogram (Table II and Fig. 2), constructed based on the similarity matrix data. As depicted in Table II, all mutants exhibit high similarity, with the A50 mutant displaying the lowest similarity value of 0.746. In contrast, the highest similarity value of 0.843 is observed in the A51 mutant. Fig. 2 illustrates that the A51 mutant bears the closest genetic distance to Adan rice. Furthermore, mutants 93.1 and 93.2 remain within this group, albeit positioned differently from the A51 mutant. Despite the A27 mutant's high similarity value with Adan rice (0.827), its position in the dendrogram is the furthest from Adan rice. It is worth noting that constructing this dendrogram entailed complex calculations and encompassed all samples, not solely compared to Adan rice but involving all mutants.

TABLE II
THE SIMILARITY MATRIX FOR 9 SAMPLES OF ADAN RICE USING 4,606 SNP MARKERS

	A27	**A28**	**A25**	**A50**	**A88**	**Adan**	**A51**	**A93.1**	**A93.2**
A27	1.000								
A28	0.857	1.000							
A25	0.863	0.879	1.000						
A50	0.810	0.807	0.803	1.000					
A88	0.810	0.811	0.798	0.855	1.000				
Adan	**0.820**	**0.785**	**0.792**	**0.746**	**0.770**	1.000			
A51	0.797	0.798	0.807	0.814	0.833	**0.843**	1.000		
A93.1	0.777	0.798	0.809	0.803	0.833	**0.826**	0.867	1.000	
A93.2	0.780	0.799	0.812	0.803	0.836	**0.817**	0.862	0.974	1.000

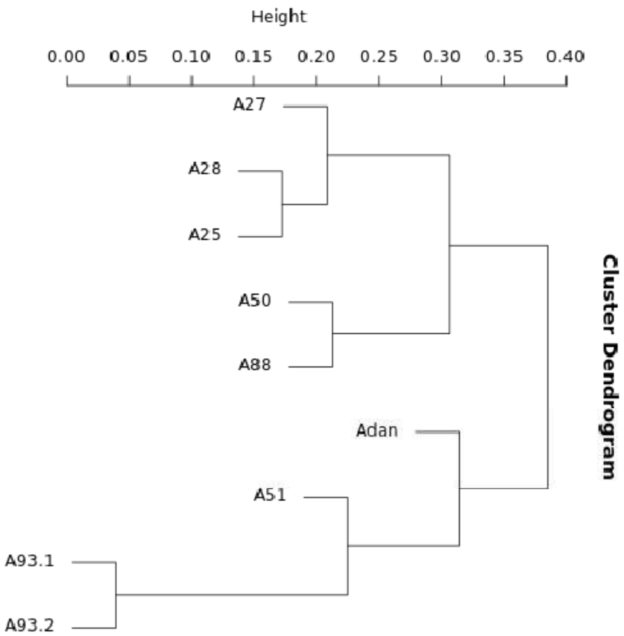


Fig. 2 Dendrogram for 9 samples of Adan rice using 4,606 SNP markers.

Based on Fig. 2, there are two groups, namely group I: mutants A27, A28, A25, A50, and A88, and group II: mutants A51, A93.1, and A93.2. Three mutants in group II have molecular closeness to the Adan rice parent. The three mutants were obtained at a dose of 150 Gy (Table I), not 100 Gy. Theoretically, the genomic damage at the 150 Gy dose should be more than the 100 Gy dose, so the 100 Gy mutant should be more closely related to the rice parent Adan. However, in this study, the higher dose resulted in a mutant whose genome was closer to that of the Adan rice.

Several researchers have utilized SNP markers to analyze genetic diversity. For instance, Aesomnuk et al. [27] employed 75 PCR-based SNP markers to assess 365 rice accessions from Thailand. Similarly, Choudhury et al. [28] utilized 30 hypervariable SSR markers and 32,782 SNP markers to investigate the genetic diversity of 298 Indian landrace rice varieties. Additionally, Huang et al. [29] employed SNP arrays comprising 44,263 markers to analyze 154 rice accessions from Yunnan province, China. These studies underscore the effectiveness of employing many SNP markers in rice kinship analysis.

Further investigation revealed mutations across all chromosomes, primarily point mutations, wherein the nitrogen bases in the mutants differ from those in Adan rice. Notably, chromosome 1 exhibited the highest frequency of mutations, while chromosome 10 displayed the lowest (Table III). Despite the stochastic nature of mutations induced by gamma rays, SNP markers effectively demonstrate the occurrence of point mutations. Among the mutants, A28 exhibited the highest number of mutations, contrasting with the A51 mutants, which displayed the fewest mutations. This finding aligns with the dendrogram (Fig. 2), indicating that the A51 mutant bears the closest similarity to Adan rice. Fig. 3 illustrates examples of alleles displaying mutations in each mutant, focusing on chromosome 1, which exhibits the highest mutation incidence compared to other chromosomes. A similar incident also happened in the research of Jankowicz-Cieslak et al. [30], where whole genome sequencing was used to detect the effects of mutations in rice. The results showed that eleven mutants had altered genomic composition.

TABLE III

THE NUMBER OF MUTATION POINTS FOR EACH CHROMOSOME IN THE ADAN RICE MUTANTS

Mutants	Chr 1	Chr 2	Chr 3	Chr 4	Chr 5	Chr 6	Chr 7	Chr 8	Chr 9	Chr 10	Chr 11	Chr 12	Total
A28	74	86	51	98	45	82	78	72	42	53	84	41	806
A88	89	71	69	84	42	96	30	84	51	54	56	49	775
A50	93	86	84	50	29	79	61	79	60	41	68	41	771
A25	89	36	69	55	43	73	81	55	59	57	84	34	735
A27	82	44	28	88	12	74	47	64	53	20	77	40	629
A93.1	97	74	61	39	37	29	70	60	25	10	46	55	603
A93.2	89	76	65	39	41	29	73	58	26	10	46	49	601
A51	53	60	48	14	34	85	74	48	1	32	46	35	530
Average	83.25	66.63	59.38	58.38	35.38	68.38	64.25	65	39.63	34.63	63.38	43	681.25
Percent*	1.81	1.45	1.29	1.27	0.77	1.48	1.40	1.41	0.86	0.75	1.38	0.93	14.79

*Percent to the total of SNP (4,606).

B. *Agronomic* analysis

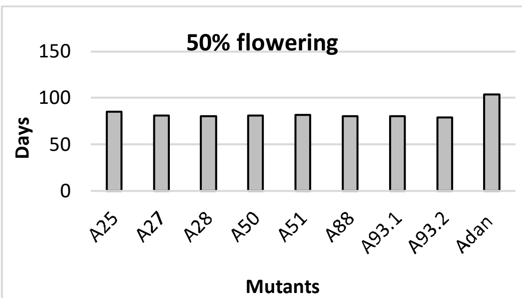
The mutant lines showed various agronomical characteristics. All mutants have shorter heading dates, but, in terms of other agronomical characters, they showed different performances. Beneficial effects were found in the lower plant height, higher panicle length, grain weight, and yield potential. Figures 3 illustrate mutant plants' field expressions and Adan rice mutants' grain morphology, respectively. Key traits such as heading date, plant height, number of productive tillers, panicle length, grain weight per hill, and yield potential are presented for each mutant. The primary aim of this study was to develop Adan rice mutants with an early heading date and characteristics resembling those of Adan rice in appearance and taste. Consequently, the initial selection of Adan rice mutants for field planting (at Muara and Krayan) prioritized those with early maturation. Selected mutants exhibited a shorter growth period (early flowering and maturity) than Adan rice (Fig. 3A). The 50% heading date for mutants ranged from 79 to 85 days.

In contrast, Adan rice typically heads in 104 days. Mutant maturity was estimated at around 109 days, compared to Adan rice's approximate 134 days to maturity. Notably, these estimates pertain to conditions in Bogor, characterized by higher temperatures than in Krayan. To mature, adan rice in Krayan requires around 5-5.5 months (150-165 days). Hence, a 109-day (4-month) growth period would enable Adan rice to be cultivated twice annually in Krayan. Reduction in maturity duration is standard in gamma-ray-induced rice mutants [31], with flowering days reduced by 4.94% to 21.40% compared to their predecessors. In this study, Adan rice mutants exhibited a reduction in flowering days ranging from 24.04% to 29.81%.

Changes in heading date among Adan rice mutants also influenced other agronomic parameters, such as plant height, number of productive tillers, and panicle length (refer to Fig. 3B, 3C, and 3D). Regarding plant height, Adan rice mutants varied from 114.1 to 144.82 cm, while Adan rice typically reached 129.45 cm. A quicker flowering period generally correlates with reduced rice plant height. Among the eight mutants studied, only A25 mutants exhibited greater height than their predecessors, while the remaining seven mutants displayed shorter stature. This reduction averaged approximately 5.43 cm (4.19%), according to Andrew-Peter-Leon et al. [32], gamma radiation-induced reduction in rice plant height can reach up to 40%. Lekha et al. [33] also discovered that local red rice mutants of the Kajejaya variety had shorter plant lifespans and heights. This demonstrates how strongly gamma rays affect these two variables.

Regarding the number of productive tillers, Adan rice mutants ranged from 10.80 to 17.00, while Adan rice typically exhibited approximately 10.30 tillers. This enhancement averaged approximately 2.34 (22.69%). Regarding panicle length, Adan rice mutants varied from 21.16 to 27.495 cm, while Adan rice typically had a length of around 26.44 cm. This reduction averaged 2.95 cm (11,17%). Mutant deviations from parental traits can vary, with some mutants displaying smaller or larger deviations. Notably, the A25 mutant exhibited the most significant deviations in plant height, number of productive tillers, and panicle length. This observation corresponds with the dendrogram, which clusters mutant A25 separately from Adan rice, with approximately 735 mutation points observed.

A



B

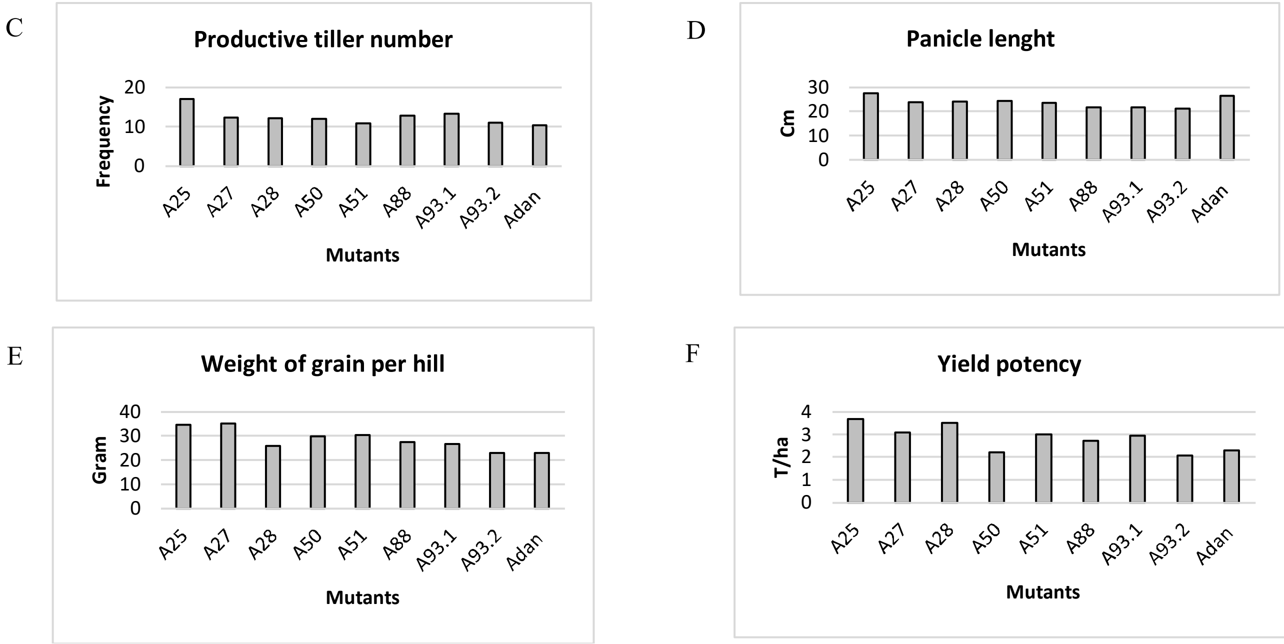


Fig. 3 Agronomic performance of Adan rice mutants.

The mutation event in Adan rice surprisingly yielded beneficial effects on yield (refer to Fig. 3E, 3F). This positive outcome was evident in seven mutants, while one mutant (A93.2) exhibited lower yields in both grain weight per hill and yield potential. Regarding grain weight per hill, Adan rice mutants ranged from 22.97 to 35.03 g, compared to Adan rice's average of approximately 22.94 g. This enhancement averaged approximately 8.23 g (27.14%). Regarding yield potential, Adan rice mutants displayed a 2.06 to 3.69 t/ha range. This enhancement averaged approximately 0.61 t/ha (26.64%). Notably, mutants A25, A27, and A28 exhibited potential yields exceeding 3 t/ha, and mutants closest to Adan rice (A51) displayed higher yields than Adan rice itself. It presents promising prospects for Adan rice development in Krayan, offering early maturation alongside high yields, which could substantially enhance farmers' income in the region, both seasonally and annually. Yield increases are occasionally observed in rice mutants resulting from gamma-ray radiation ([34], [31]. Kato [4] reported a yield increase of approximately 22.5% in gamma-ray radiation-induced mutants of Fukuhibiki rice (FukuhibikiH6 and FkuhibikiH8) compared to the original parent.

C. Rice Grain Quality Test

In addition to yield, the taste quality of rice has become increasingly significant as consumer preference for flavorful rice rises. Numerous breeding endeavors have been undertaken by researchers to enhance the taste of rice [35], [36], [37]. Adan rice is already renowned for its excellent taste. Hence, any resulting mutants must retain this characteristic to ensure acceptance by the local community.

Milling quality is a pivotal aspect of rice grain quality, with the Indonesian National Standard using it as a benchmark for grain classification. The premium and top-tier classifications hinge on high-head rice yield, whiteness, transparency, and minimal levels of broken, yellow, and chalky rice. According to this classification, most mutants fell into lower classes than Adan rice, with only three mutants showing minimal deviation. Nonetheless, physical quality is heavily influenced by environmental factors. Physical damage to kernels increases susceptibility to breakage during dehulling and milling, consequently reducing head rice yields. Various factors across rice production, from cultivation to storage, can impact quality outcomes. The grain quality of Adan rice can be seen in Table IV.

Overall, the mutants exhibited lower milling quality, evident from the reduced percentage of head rice, indicating a higher proportion of broken and brewer rice. Head rice percentages ranged from 26.59% to 90.60%, averaging 60.16%, markedly lower than Adan rice, which boasted 88% head rice. Nevertheless, three mutants demonstrated relatively strong milling performance: A28, A27, and A25, with head rice percentages of 90.60%, 75.95%, and 75.76%, respectively. Visually, mutants displayed lower whiteness levels (averaging 50.12%) compared to Adan rice (55.6%) but exhibited much higher transparency (56.67% versus Adan rice's 21.67%). However, mutants showcased superior performance in yellowing and chalkiness, averaging 0.40% and 0.045%, respectively, compared to Adan rice's 0.19% and 0.17%. Both mutants and Adan rice exhibited zero dockage and low moisture content. Wang et al. [38] reported increased chalky levels in OsbZIP09 rice mutants, attributed to loosening in the ventral area of the endosperm, resulting in the release of chalkiness typically localized in that area.

Amylose content, gelatinization temperature, and gel consistency are three pivotal parameters influencing rice cooking and eating quality [35]. Regarding amylose content, the mutants exhibited significantly higher levels (25.73%) than Adan rice (17.83%). Most mutants displayed amylose content exceeding 20%, with only two mutants, A25 and A28, falling below this threshold. According to amylose levels, these two mutants, along with Adan rice, are classified as very fluffy, while other mutants are categorized as fluffier or less fluffy based on the SNI 6128:2015 classification. Occasionally, gamma rays can elevate amylose levels [39], potentially resulting in less fluffy rice, as higher amylose content tends to reduce fluffiness. Adan rice, with an amylose content below 20%, retains its fluffy texture. According to Ronie et al. [40], Bario rice, the identical twin of Adan rice, has an amylose content of up to 26.67%, meaning that Adan rice is fluffier than Bario rice. Even though in Malaysia, Adan rice is frequently referred to as Bario.

Observation of other eating/cooking quality characteristics revealed some distinctions between the mutants and Adan rice. The mutants exhibited nearly identical gelatinization times (32 min) compared to Adan rice (30 min). However, mutants displayed higher viscosities across all temperature measurements—50°C, 90°C, at 20 minutes of 93°C, and the peak—with peak times of relatively similar duration. On average, mutant viscosity levels surpassed those of Adan rice, measuring 356 compared to 320, 343 compared to 320, 272 compared to 240, and 657 compared to 350 for peak viscosity, viscosity at 93°C, viscosity at 20 minutes of 93°C, and viscosity at 50°C, respectively. The Setback viscosity analysis indicated that three mutants exhibited higher levels, while five showed lower viscosity levels in comparison.

On average, the mutant exhibited slightly higher lipid and protein content, marginally lower carbohydrate content, and increased amylose content. Specifically, four mutants displayed higher lipid content, while four others exhibited lower. The mutants, on average, demonstrated significantly higher protein content (8.30%) than Adan (7.18%), except for mutant A93.2. Moreover, their carbohydrate content was marginally lower (81.1%) than Adan's (83%). It is worth noting that besides protein and lipid levels, iron, zinc, and vitamin A levels are crucial for addressing stunting issues prevalent in rural areas like the Krayan district [41]. However, these measurements were not used in this study.

Viscosity, an essential cooking quality, is measured by analyzing an aqueous suspension of ground rice flour using the Rapid Visco Analyzer (RVA). This instrument assesses the pasting quality of rice, which can vary significantly across different varieties. Parameters such as peak viscosity (maximum viscosity during heating), trough (minimum viscosity after the peak), final viscosity (FV), and their interplay offer insights into the pasting properties of the flour sample. Setback, calculated as the difference between final and peak viscosity, indicates cooked rice firmness, with higher values indicating a firmer texture. Lu et al. [42] reported that low paste viscosity corresponds to low amylose content and a soft gel, indicating superior eating quality. In examining Adan rice and its mutants, three mutants exhibited higher setback viscosity levels, while five others showed slightly lower levels. It suggests that the mutants possess a pasting quality comparable to the parent variety.

Regarding cooking quality, mutants A25 and A28 exhibit favorable traits with low amylose content and reduced set-back viscosity, indicating excellent cooking performance. Furthermore, these mutants possess higher lipid and protein content and a pleasing aroma—attributes reminiscent of the widely preferred Adan rice. Adan rice is renowned for its fluffy texture and delightful aroma, both during cultivation and after harvesting. These promising characteristics position the two mutants as potential candidates for release as new varieties. However, before their release, thorough testing in the Krayan region, with the involvement of local communities, is essential to validate their eating quality.

Research on Adan rice is advancing by entrusting selected Adan rice mutants to local farmers, particularly those who do not migrate for work in Malaysia. These mutants are cultivated on their lands following Adan rice plantations. With the completion of road access to Nunukan Regency through the Kayan Mentarang National Park and an anticipated rise in demand, Adan rice could be cultivated twice a year to meet consumer needs. Increased demand for this prized variety would make the availability of these mutants highly advantageous. Aside from that, the Krayan community's economy greatly benefits from the role [43].

TABLE IV
THE RESULT OF THE GRAIN QUALITY TEST FOR ADAN RICE MUTANTS

No.	Type of analysis	Method	Unit	Samples								
				A25	A27	A28	A50	A51	A88	A93.1	A93.2	Adan
1	Head rice	SNI-6128-2008	%	75.76	75.95	90.60	59.67	66.89	35.46	50.36	26.59	88.17
2	Broken grain	SNI-6128-2008	%	23.99	23.51	9.16	31.35	32.03	53.54	40.23	58.11	4.55
3	Small brokens or "brewers rice"	SNI-6128-2008	%	0.25	0.54	0.24	8.64	1.08	10.45	8.62	13.74	1.07
4	Yellow grain	SNI-6128-2008	%	0.73	0.84	0.29	0.60	0.26	0.15	0.18	0.13	0.19
5	Chalkiness	SNI-6128-2008	%	0.00	0.25	0.00	0.00	0.11	0.00	0.00	0.00	0.17
6	Red grain	SNI-6128-2008	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	Grain content	SNI-6128-2008	%	0.00	0.00	0.00	0.33	0.00	0.54	0.79	1.56	6.20
8	Dockage	SNI-6128-2008	%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
9	Polite content	SNI-6128-2008	%	100.00	100.00	100.00	90.00	100.00	90.00	90.00	90.00	80.00
10	Whiteness	SNI-6128-2008	%	48.28	45.08	43.95	52.19	42.53	57.24	55.16	56.52	56.94
11	Transparency	SNI-6128-2008	%	37.78	47.08	54.86	56.25	48.06	70.56	72.22	66.53	21.67
12	Moisture content	Gravimetry	%	9.45	9.35	9.82	6.53	8.96	7.50	7.63	6.69	8.01
13	Ash content	Gravimetry	%	0.50	2.72	0.66	0.49	0.61	0.57	0.76	0.47	0.78
14	Lipid content	Soxhlet	%	1.74	2.56	1.92	0.70	2.55	0.86	0.92	0.73	1.49
15	Protein content	Kjeldahl	%	8.50	9.46	8.97	7.30	9.19	7.28	8.59	7.12	7.18
16	Carbohydrate content	*By Different*	%	79.81	75.91	78.63	84.98	78.69	83.79	82.10	84.99	82.54
17	Amylose content	Spectro	%	19.29	23.53	19.26	27.19	24.49	33.03	30.56	28.52	17.83
18	Gelatinization time		Minute	31.00	31.00	33.00	34.00	30.00	33.00	34.00	33.00	30.00
19	Peak time		Minute	41.00	41.00	40.00	42.00	39.00	42.00	43.00	42.00	42.00
20	Peak viscosity		BU	420.00	360.00	340.00	310.00	330.00	380.00	320.00	390.00	320.00
20	Viscosity 93°C		BU	400.00	350.00	310.00	310.00	300.00	380.00	310.00	390.00	320.00
22	Viscosity 93°C/20'		BU	310.00	200.00	310	210.00	300.00	290.00	260.00	300.00	240.00
23	Viscosity 50°C		BU	680.00	450.00	680.00	510.00	660.00	770.00	710.00	800.00	350.00
24	Set back viscosity		BU	(+) 370	(+) 371	(+) 373	(+) 300	(+) 376	(+) 480	(+) 420	(+) 500	(+) 378

SNI = Standar Nasional Indonesia (=Indonesian National Standard), BU = Brabender Unit.

IV. Conclusion

SNP markers serve as reliable tools for distinguishing mutants from Adan rice parents. Based on SNP analysis, the mutant lines of Adan rice were categorized into two groups: group I comprising mutants A27, A28, A25, A50, and A88, and group II comprising mutants A51, A93.1, and A93.2. Notably, all Adan rice mutants exhibit a shorter heading date than their parent, with the highest yield increase reaching 161.14%. Among these mutants, A25 and A28 have several beneficial characteristics and minimal defects compared to the parent variety. Efforts to introduce Adan rice mutants to residents remain necessary to ensure acceptance, particularly as these mutants share similar traits with Adan rice but offer the advantage of being cultivable twice a year.

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References

[1] F. Fauziah and M. Rizal, "Potensi Pengembangan Padi Adan di Kabupaten Nunukan," *Proceedings Series on Physical & Formal Sciences*, vol. 4, pp. 90–94, Nov. 2022, doi: 10.30595/pspfs.v4i.488.

[2] Rahmianti, "Beras Adan, si beras Krayan yang mendunia," [Online]. Available: https://www.rri.co.id/daerah/468621/beras-adan-si-beras-krayan-yang-mendunia.

[3] H. Wei, X. Wang, H. Xu, and L. Wang, "Molecular basis of heading date control in rice," *BIOTECH*, vol. 1, no. 4. Springer, pp. 219–232, Oct. 01, 2020. doi: 10.1007/s42994-020-00019-w.

[4] H. Kato, F. Li, and A. Shimizu, "The selection of gamma-ray irradiated higher yield rice mutants by directed evolution method," *Plants*, vol. 9, no. 8, pp. 1–16, Aug. 2020, doi: 10.3390/plants9081004.

[5] A. Abdelnour-Esquivel, J. Perez, M. Rojas, W. Vargas, and A. Gatica-Arias, "Use of gamma radiation to induce mutations in rice (*Oryza sativa* L.) and the selection of lines with tolerance to salinity and drought," *In Vitro Cellular and Developmental Biology - Plant*, vol. 56, no. 1, pp. 88–97, Feb. 2020, doi: 10.1007/s11627-019-10015-5.

[6] A. A. Ali, O. A. Galal, A. M. Elmoghazy, and M. M. Abd-Elrazek, "Using gamma rays for genetic improvement of rice resistance to blast disease," *Journal of Agricultural Chemistry and Biotechnology*, vol. 13, no. 10, pp. 85–90, Oct. 2022, doi:10.21608/jacb.2022.152986.1028.

[7] B. Saragih, N. M. Naibaho, and B. Saragih, "Nutritional, functional properties, glycemic index and glycemic load of indigenous rice from North and East Borneo," *Food Res*, vol. 3, no. 5, pp. 537–545, Oct. 2019, doi: 10.26656/fr.2017.3(5).035.

[8] S. G. Hwang *et al.*, "Resequencing of a core rice mutant population induced by gamma-ray irradiation and its application in a genome-wide association study," *Journal of Plant Biology*, vol. 63, no. 6, pp. 463–472, Dec. 2020, doi: 10.1007/s12374-020-09266-2.

[9] C. Lee *et al.*, "Development and application of a target capture sequencing SNP-genotyping platform in rice. ," *Genes* , vol. 13, no. 794, Apr. 2022, doi: 10.3390/genes13050794.

[10] K. Y. Morales *et al.*, "An improved 7K SNP array, the C7AIR, provides a wealth of validated SNP markers for rice breeding and genetics studies," *PLoS One*, vol. 15, no. 5, May 2020, doi:10.1371/journal.pone.0232479.

[11] C. Zhang *et al.*, "Rice3K56 is a high-quality SNP array for genome-based genetic studies and breeding in rice (*Oryza sativa* L.)," *Crop Journal*, vol. 11, no. 3, pp. 800–807, Jun. 2023, doi:10.1016/j.cj.2023.02.006.

[12] K. W. Kim, B. Nawade, J. Nam, S. H. Chu, J. Ha, and Y. J. Park, "Development of an inclusive 580K SNP array and its application for genomic selection and genome-wide association studies in rice," *Front Plant Sci*, vol. 13, 1036177, Oct. 2022, doi:10.3389/fpls.2022.1036177.

[13] P. M. Babu *et al.*, "Stable SNP allele associations with high grain zinc content in polished rice (*Oryza sativa* L.) identified based on ddRAD sequencing," *Front Genet*, vol. 11, Aug. 2020, doi:10.3389/fgene.2020.00763.

[14] J. W. Lee, J. S. Oh, and S. C. Yoo, "Development of SNP marker set to select varieties tolerant to multiple abiotic stresses in rice," *Plant Breed Biotechnol*, vol. 11, no. 3, pp. 208–219, 2023, doi:10.9787/pbb.2023.11.3.208.

[15] E. Sales, J. García-Romeral, and C. Domingo, "Bioinformatics approach for developing a minimum set of SNP markers for identification of temperate japonica rice varieties cultivated in Spain," *PLoS One*, vol. 18, no. 6, e0286839, June, Jun. 2023, doi:10.1371/journal.pone.0286839.

[16] C. Winarti *et al.*, "Nutrient composition of Indonesian specialty cereals: rice, corn, and sorghum as alternatives to combat malnutrition," *Prev Nutr Food Sci*, vol. 28, no. 4, pp. 471–482, Dec. 2023, doi: 10.3746/pnf.2023.28.4.471.

[17] BSN, *Standar Nasional Indonesia (SNI): Beras (= National Standard Bureau: Rice)*. Jakarta: Badan Standardisasi Nasional, 2015.

[18] A. N. M. R. Bin Rahman and J. Zhang, "Trends in rice research: 2030 and beyond," *Food and Energy Security*, vol. 12, no. 2, John Wiley and Sons Inc, Mar. 01, 2023. doi: 10.1002/fes3.390.

[19] D. Gong *et al.*, "Genetic improvements in rice grain quality: A review of elite genes and their applications in molecular breeding," *Agronomy*, vol. 13, no. 5, 1375, MDPI, May 01, 2023. doi:10.3390/agronomy13051375.

[20] M. J. Thomson *et al.*, "Large-scale deployment of a rice 6K SNP array for genetics and breeding applications," *Rice*, vol. 10, no. 40, Dec. 2017, doi: 10.1186/s12284-017-0181-2.

[21] S. L. Dellaporta, J. Wood, and J. B. Hicks, "A plant DNA minipreparation: Version II," *Plant Mol Biol Report*, vol. 1, no. 4, pp. 19–21, 1983.

[22] Y. Du *et al.*, "Frequency and spectrum of mutations induced by gamma rays revealed by phenotype screening and whole-genome re-sequencing in *Arabidopsis thaliana*," *Int J Mol Sci*, vol. 23, no. 654, Jan. 2022, doi: 10.3390/ijms23020654.

[23] L. Ma, F. Kong, K. Sun, T. Wang, and T. Guo, "From classical radiation to modern radiation: Past, present, and future of radiation mutation breeding," *Frontiers in Public Health*, vol. 9, :768071, Frontiers Media S.A., Dec. 21, 2021. doi:10.3389/fpubh.2021.768071.

[24] R. Baadu, K. P. Chong, J. A. Gansau, M. R. M. Zin, and J. Dayou, "A systematic review on physical mutagens in rice breeding in Southeast Asia," *PeerJ*, vol. 11, no. 5, e15682 2023, doi: 10.7717/peerj.15682.

[25] A. Jadhav *et al.*, "Assessment of genetic variability in gamma rays induced mutants of rice (*Oryza sativa* L.)," vol. 12, no. 6, pp. 4417–4422, 2023.

[26] J. Prasetiyono, T. Rafsanjani, T. Aminingsih, Tasliah, and S. Moeljaopawiro, "Genetic variation of Adan, a Krayan local rice mutant, using microsatellite markers," in *The Second International Conference on Genetic Resources and Biotechnology, Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture*, I. Tasma, D. Utami, I. Roostika, Y. Suryadi, Chaerani, E. Riyanti, P. Lestari, T. Hadiarto, Reflinur, J. Prasetiyono, Fatimah, S. Diantina, T. Priyatno, K. Kusumanegara, W. Enggarini, R. Terryana, and D. Satyawan, Eds., Jakarta: AIP Conference Proceedings 2462, 2022, doi: 10.1063/5.0075660.

[27] W. Aesomnuk *et al.*, "Estimation of the genetic diversity and population structure of thailand's rice landraces using snp markers," *Agronomy*, vol. 11, no. 995, 2021, doi: 10.3390/agronomy11050995.

[28] D. R. Choudhury *et al.*, "SSR and SNP marker-based investigation of Indian rice landraces in relation to their genetic diversity, population structure, and geographical isolation," *Agriculture (Switzerland)*, vol. 13, no. 4, 823, Apr. 2023, doi: 10.3390/agriculture13040823.

[29] P. Huang *et al.*, "Genetic analysis of a collection of rice germplasm (*Oryza sativa* L.) through high-density SNP array provides useful information for further breeding practices," *Genes (Basel)*, vol. 13, no. 830, May 2022, doi: 10.3390/genes13050830.

[30] J. Jankowicz-Cieslak *et al.*, "Spectrum and density of gamma and X-ray induced mutations in a non-model rice cultivar," *Plants*, vol. 11, no. 23, 3232, Dec. 2022, doi: 10.3390/plants11233232.

[31] R. Sao, P. K. Sahu, R. S. Patel, B. K. Das, L. Jankuloski, and D. Sharma, "Genetic improvement in plant architecture, maturity duration and agronomic traits of three traditional rice landraces through gamma ray-based induced mutagenesis," *Plants*, vol. 11, no. 3448, Dec. 2022, doi: 10.3390/plants11243448.

[32] M. T. Andrew-Peter-Leon, S. Ramchander, K. K. Kumar, M. Muthamilarasan, and M. A. Pillai, “Assessment of efficacy of mutagenesis of gamma-irradiation in plant height and days to maturity through expression analysis in rice,” *PLoS One*, vol. 16, no. 1, e0245603, Jan. 2021, doi: 10.1371/journal.pone.0245603.

[33] Lekha P. S., A. J. Shridevi, Nachiketha T. K., D. Kumar, B. Halingali I., and Jayashree S., “Genetic analysis of M_5 generation of gamma irradiated red rice (*Oryza sativa* L.) mutant lines,” *International Journal of Environment and Climate Change*, vol. 14, no. 3, pp. 355–363, Mar. 2024, doi: 10.9734/ijecc/2024/v14i34047.

[34] W. N. Hanifah, Parjanto, S. Hartati, and A. Yunus, “The performances of M4 generation of mentik susu rice mutants irradiated with gamma-ray,” *Biodiversitas*, vol. 21, no. 9, pp. 4041–4046, Sep. 2020, doi:10.13057/biodiv/d210915.

[35] A. Zhang *et al.*, “Genetic analysis for cooking and eating quality of super rice and fine mapping of a novel locus qGC10 for gel consistency,” *Front Plant Sci*, vol. 11, no. 342, pp. 1–9, March 2020, doi: 10.3389/fpls.2020.00342.

[36] L. Huang *et al.*, “Improving rice eating and cooking quality by coordinated expression of the major starch synthesis-related genes, SSII and Wx, in endosperm,” *Plant Mol Biol*, vol. 106, no. 4–5, pp. 419–432, Jul. 2021, doi: 10.1007/s11103-021-01162-8.

[37] M. S. Kim *et al.*, “Breeding of high cooking and eating quality in rice by marker-assisted backcrossing (Mabc) using kasp markers,” *Plants*, vol. 10, no. 4, pp. 1–18, 2021, doi: 10.3390/plants10040804.

[38] C. X. Wang *et al.*, “Grain quality and starch physicochemical properties of chalky rice mutant,” *Agronomy*, vol. 11, no. 8, Aug. 2021, doi: 10.3390/agronomy11081575.

[39] R. P. Bachtari, S. Listyawati, and Sutarno, “ Starch, amylose and amylopectin levels of M_5 and M_6 generations of black rice irradiated by gamma Co 60 ray ,” *J Phys Conf Ser*, vol. 1436, p. 012116, Jan. 2020, doi: 10.1088/1742-6596/1436/1/012116.

[40] M. E. Ronie, A. H. Abdul Aziz, N. Q. I. Mohd Noor, F. Yahya, and H. Mamat, “Characterisation of Bario rice flour varieties: Nutritional compositions and physicochemical properties,” *Applied Sciences (Switzerland)*, vol. 12, no. 18, Sep. 2022, doi: 10.3390/app12189064.

[41] S. Sultana, M. Faruque, and M. R. Islam, “Rice grain quality parameters and determination tools: a review on the current developments and future prospects,” *International Journal of Food Properties*, vol. 25, no. 1. Taylor and Francis Ltd., pp. 1063–1078, 2022. doi: 10.1080/10942912.2022.2071295.

[42] Z. Lu *et al.*, “Grain quality characteristics analysis and application on breeding of Yuenongsimiao, a high-yielding and disease-resistant rice variety,” *Sci Rep*, vol. 13, no. 1, Dec. 2023, doi: 10.1038/s41598-022-21030-9.

[43] K. Khaerunnisa *et al.*, “Is agricultural institutions affect the sustainability of local Adan rice farming?,” *Indigenous Agriculture*, vol. 1, no. 1, pp. 18–28, Mar. 2023, doi: 10.20956/ia.v1i1.25785.