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Genetic Diversity of Garut Mutant (*Maranta arundinacea* L.) (M3) with Random Amplified Polymorphic DNA (RAPD) Analysis

(Kepelbagaian Genetik Mutan Garut (*Maranta arundinacea* L.) (M3) dengan Analisis DNA Polimorfik Diperkuat Rawak (RAPD))

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ABSTRACT

Arrowroot is a type of tuber that is rich in carbohydrates. This plant can grow well in various parts of Indonesia. However, arrowroot has a low level of genetic diversity, making it difficult to obtain superior varieties with high productivity. Mutation treatment with gamma-ray radiation is expected to increase the diversity of arrowroot to produce superior-quality plants. This study aimed to determine the genetic diversity of arrowroot mutants resulting from the 4th generation of gamma-ray radiation (M3). The research analysed molecular markers using Random Amplified Polymorphic DNA (RAPD). A total of 30 samples of arrowroot M3 DNA were amplified using 11 selected primers. The results of the analysis show that there are six specific primers selected from the selection, which have the potential to produce high polymorphism bands that can be used for diversity analysis. The similarity index of arrowroot mutant is 0.69 to 0.99, which means that it still has a high similarity between arrowroot mutant. Based on genetic similarity of 84%, mutant arrowroot (M3) can be divided into 7 clusters.

Keywords: DNA; Garut mutant; genetic diversity; RAPD

ABSTRAK

Ubi garut adalah sejenis ubi yang kaya dengan karbohidrat. Tumbuhan ini boleh tumbuh dengan baik di pelbagai tempat di Indonesia. Walau bagaimanapun, ubi garut mempunyai tahap kepelbagaian genetik yang rendah, menjadikannya sukar untuk mendapatkan varieti unggul dengan produktiviti tinggi. Rawatan mutasi dengan sinaran sinar gamma dijangka meningkatkan kepelbagaian anak panah untuk menghasilkan tumbuhan berkualiti tinggi. Kajian ini bertujuan untuk menentukan kepelbagaian genetik mutan ubi garut yang terhasil daripada sinaran sinar gamma (M3) generasi ke-4. Penyelidikan menganalisis penanda molekul menggunakan DNA Polimorfik Diperkuat Rawak (RAPD). Sebanyak 30 sampel DNA M3 anak panah telah dikuatkan menggunakan 11 primer terpilih. Hasil analisis menunjukkan terdapat enam primer khusus yang dipilih daripada pemilihan yang berpotensi menghasilkan jalur polimorfisme tinggi yang boleh digunakan untuk analisis kepelbagaian. Indeks kesamaan mutan ubi garut adalah 0.69 hingga 0.99, yang bermaksud ia masih mempunyai persamaan yang tinggi antara mutan ubi garut. Berdasarkan persamaan genetik sebanyak 84%, anak panah mutan (M3) boleh dibahagikan kepada 7 kelompok.

Kata kunci: DNA; kepelbagaian genetik; mutan garut; RAPD

INTRODUCTION

The diversity of alternative food crops in Indonesia is relatively abundant. One of the plants that the people of Indonesia can consume as an alternative food crop is tubers. Various types of tubers that are rich in carbohydrates can grow in Indonesian soil. One example of a tuber that can grow well in Indonesia is the Garut tuber (*Maranta arundinacea* L.). The arrowroot plant comes from South America, the Caribbean, and Mexico (Lim 2016). Arrowroot plants flourish in hot and humid climates where

the best temperature for growth is around 20-30 °C (Reddy 2015). Arrowroot plants are very welcome in Indonesia for their many benefits. Arrowroot can be used as a primary material for making bioethanol, which is an alternative renewable fuel (Effendi & Palupi 2013). In addition, arrowroot also has the potential to be a raw material for pharmaceuticals, textiles, pulp paper-making, bioenergy, packaging, automotive, and many other industries (Tarique et al. 2021).

The bulbs are the most widely used part of the arrowroot plant. According to Lim (2016), 100 g of arrowroot tubers provide 125 calories of energy, with a composition of 67.4 g of moisture, 1.7 g of protein, 0.2 g of fat, 29.5 g of total carbohydrates, 2.0 g of dietary fiber, 1.2 g of ash, and specific mineral content including 15 mg of calcium, 18 mg of phosphorus, and 1.9 mg of iron. Furthermore, the tubers contain essential vitamins such as 0.13 mg of thiamin, 0.02 mg of riboflavin, 0.5 mg of niacin, and 7 mg of ascorbic acid. Moreover, Madineni et al. (2012) highlight that arrowroot also contains amylose maranta starch, constituting 24.8% of the tuber composition, falling within the typical range of 18-30%. Additionally, the tubers are a source of various minerals including phosphorus, calcium, magnesium, potassium, iron, manganese, and zinc, which contribute to the nutritional value of arrowroot. These nutritional components make arrowroot a valuable dietary resource, providing essential nutrients and energy. The presence of macronutrients like carbohydrates and dietary fibre, along with micronutrients such as calcium, phosphorus, and iron, underscores the potential health benefits of incorporating arrowroot into the diet. Furthermore, the presence of vitamins like thiamin, riboflavin, niacin, and ascorbic acid further enhances the nutritional profile of arrowroot, making it a well-rounded food source.

The many benefits that can be obtained from arrowroot plants indicate the need to develop superior accessions that increase arrowroot production yields. Superior accessions for arrowroot can be derived from germplasm in nature. However, superior accession can be pursued through plant breeding programs using the cross method or utilising technology such as genetic mutations (Suhartini & Hadiatmi 2016). Genetic mutation is one way that can be taken to increase genetic diversity in plants. The fundamental concept of genetic mutation involves altering the genetic composition of an individual (Sobrizal 2016). Induction is done by using mutagens such as gamma ray radiation which can be applied to the desired seeds or plant parts.

Mutational plant breeding effectively changes a few traits and improves plant accessions (Sobrizal 2016). Physical mutations have been carried out by Deswina, Prihastuti and Saputra (2019) who give gamma rays at a dose of 10 Gray to 50 Gray, interval 10. The mutation results affect the morphology of arrowroot plants, such as shoot growth and stem height. Research conducted by Meliala, Basuki and Seogianto (2016) showed that the administration of gamma rays with different doses on the phenotypic properties of upland rice (*Oryza sativa*) had a significant effect that reached a 99% confidence level as well as on its genetic characteristics, resulting in high genetic diversity resulted in obtaining superior nature and character.

The genetic diversity of the arrowroot can be analysed using the Random Amplified Polymorphic DNA or RAPD technique. RAPD molecular markers are used in analysing the genetic diversity of arrowroot because they are easy to amplify and can detect polymorphic DNA fragments (Nurtjahjaningsih et al. 2015). These molecular markers can be used in the vegetative phase of plants without damaging the plants due to small sampling (Gusmiaty et al. 2016). The RAPD marker technique has been widely used to detect plant genetic diversity, such as genetic diversity and plant relationships of *Calophyllum inophyllum* (Nurtjahjaningsih et al. 2015), pine (Gusmiaty et al. 2016), teak (Widyatmoko, Rimbawanto & Chasani 2013), and Garut (Paradisa, Deswina & Mulyaningsih 2016).

The phenotypic evaluation and selection of arrowroot mutants in the previous generation has been carried out by Deswina, Prihastuti and Saputra (2019), who produced five accessions of arrowroot plants obtained from the selection of approximately 30 collections of arrowroot tubers from West Java, Central Java, and Banten. Gamma-ray radiation was applied to the five accessions with an application dose of 10 to 50 Gray. This study aims to determine plants' morphological and genetic diversity in the fourth generation (M3). M3 genetic diversity data using the RAPD marker technique is needed to improve the genetic quality of arrowroot, primarily to support the assembly of high-yielding varieties.

MATERIALS AND METHODS

The research was carried out at the Biotechnology Research Center, LIPI, in January 2020-April 2020. Currently, it is part of the National Research and Innovation Agency.

MORPHOLOGICAL CHARACTERIZATION OF M3 MUTANTS

The arrowroot plants were planted in polybag media, and a factorial randomised block design was used with two factors. The first factor is five accessions of arrowroot mutants, and the second is radiation dose. This study was repeated three times. The five accessions are the fourth generation (M3) of the Pulosari Accessions, 25 Pandeglang, Cikondang, Taman Sari, and MN-1. Gamma radiation was performed at 0, 10, 20, 30, 40, and 50 gray each as shown in Table 1. Morphological observations were made by directly observing shoot height, number of tillers, leaf length, and leaf width. The data obtained were analysed with ANOVA using the SAS program version 9.4. If F count > F table, it is further tested using Duncan's test at = 5%.

TABLE 1. Samples of arrowroot plants resulting from gamma ray

No.	Plant	Treatment	No.	Plant	Treatment
1	Pulosari (AR0)	0 gray	16	Cikondang (CR3)	30 gray
2	Pulosari (AR1)	10 gray	17	Cikondang (CR4)	40 gray
3	Pulosari (AR2)	20 gray	18	Cikondang (CR5)	50 gray
4	Pulosari (AR3)	30 gray	19	Taman Sari (DR0)	0 gray
5	Pulosari (AR4)	40 gray	20	Taman Sari (DR1)	10 gray
6	Pulosari (AR5)	50 gray	21	Taman Sari (DR2)	20 gray
7	25 Pandeglang (BR0)	0 gray	22	Taman Sari (DR3)	30 gray
8	25 Pandeglang (BR1)	10 gray	23	Taman Sari (DR4)	40 gray
9	25 Pandeglang (BR2)	20 gray	24	Taman Sari (DR5)	50 gray
10	25 Pandeglang (BR3)	30 gray	25	MN-1 (ER0)	0 gray
11	25 Pandeglang (BR4)	40 gray	26	MN-1 (ER1)	10 gray
12	25 Pandeglang (BR5)	50 gray	27	MN-1 (ER2)	20 gray
13	Cikondang (CR0)	0 gray	28	MN-1 (ER3)	30 gray
14	Cikondang (CR1)	10 gray	29	MN-1 (ER4)	40 gray
15	Cikondang (CR2)	20 gray	30	MN-1 (ER5)	50 gray

ANALYSIS OF ARROWROOT CHLOROPHYLL CONTENT

The isolation of chlorophyll from arrowroot plants was performed based on Madrigal et al. (2018) with modifications. Plant samples were taken from the oldest and fully opened arrowroot plant leaves with almost the same color and weight of 0.1 g. Next, the leaf samples were cut into small pieces and put into a 2 mL tube. The sample to be tested is stored in an ice box so that the freshness of the sample is maintained. Each sample was given 1.5 mL of 90% ethanol and incubated for 8-12 h. Then, the sample is centrifuged to separate the supernatant and the pellet. The supernatant obtained was analyzed using a Implen NanoPhotometer™ at wavelengths of 466, 649, and 470 nm. The chlorophyll variables observed included: chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, calculated based on Sumanta et al. (2014) as follows: Ch-a: 13.36 A664 – 5.19 A649; Ch-b: 27.43 A649 – 8.12 A664; and C x+c: (1000 A470 – 1.43Ca - 97.63 Cb)/209.

ANALYSIS OF ARROWROOT GENETICS

DNA isolation was carried out using the Cetyltrimethyl Ammonium Bromide (CTAB) method based on Doyle and Doyle (1990) with modification. ±0.5 g of plant leaves was put into a 1.5 mL tube, and liquid nitrogen was added. The leaves were ground to smooth, and 500 mL CTAB (50 mM Tris-HCl (pH 8), 0.7 M NaCl was added. The samples were then incubated in a water bath at 65 °C for 1 h. After incubation, 750 µL chloroform: isoamyl alcohol (24:1) was added. The sample was inverted and then centrifuged at 12,000 rpm for 13 min. The supernatant obtained was transferred to a new tube, and 400 µL of Isopropanol was added. The sample was incubated at -20 °C overnight. The sample was centrifuged for 10 min at a speed of 12,000 rpm. The supernatant obtained was discarded, and the pellet was washed using 300 µL of 70% ETOH. The sample was centrifuged for 10 min at 12,000 rpm, and the resulting supernatant was discarded. After the pellet was dried at room temperature, TE buffer pH 8 (containing RNAse) was added as much as 30 µL per tube. The samples were incubated at 37 °C for 30 min and stored at -20 °C.

There were 22 RAPD primers used, namely OPA 06, OPAB 03, OPAM 01, OPAM 03, OPAM 12, OPAM 18, OPC 01, OPD 8, OPF 07, OPG 13, OPG 18, OPH 03, OPK 05, OPS 19, OPW 05, OPW 16, OPY 08, OPZ 03, OPB 14, OPC 05, OPA 13, and OPA 02. The reaction was performed using 4.5 µL Nuclease free water (NFW), 6.25 µL Taq Polymerase (My Taq Bioline), 1 µL DNA template (100 ng/µL), and 0.75 µL primer (10 µM) with initial separation (pre-denaturation) was carried out at 95 °C for 1 min. The following steps are separation (denaturation), annealing, and elongation at 95 °C, 30-34 °C, and 72 °C for 30 s each and repeated for 45 cycles. The final elongation stage was carried out at 72 °C for 5 min. Visualisation was carried out by electrophoresis using 2% Agarose. Staining was carried out by adding 10 µL SYBR® SafeDNA Gel Stain (Invitrogen) to Agarose. Electrophoresis results were seen using GelDoc. A 1 kb marker (Invitrogen) was used as a marker.

Molecular data analysis was carried out based on the results of the DNA band scoring that appeared on the agarose. The scoring results are in the form of binary data. If there is a band, it is given a score of one (1); if there is no band, it is given a zero (0) score. Polymorphic Information Content (PIC) is used as a standard to evaluate genetic markers based on DNA bands resulting from PCR amplification (Carsono et al. 2014). PIC for RAPD is calculated using the equation Roldan-Ruiz et al. (2000), namely PIC = 2 × fi × (1-f1), where fi is the frequency of the amplified allele (band appears) and (1-fi) is the frequency of the non-amplified allele (band does not appear). According to Carsono et al. (2014), the PIC value is divided into three, namely PIC > 0.5 = very informative, then 0.25 > PIC > 0.5 = moderate, and PIC < 0.25 = low. Binary data were also analysed using the computer program NTSYS-pc version 2.1. The results of the genetic similarity analysis were carried out using the UPGMA (Unweighted Pair Method Group Arithmetic) and presented in a dendrogram.

RESULTS AND DISCUSSION

Plant mutation is a significant factor in plant breeding, genetic research, and adaptation. Mutagenesis in plants, whether induced by physical agents like ion beams (Tanaka, Shikazono & Hase 2010), gamma rays (Du et al. 2022), or through biotechnological tools like CRISPR/Cas9 (Osakabe et al. 2016), can lead to the generation of novel genetic variations. These mutations can impact various plant traits, such as herbicide resistance (Yu et al. 2010), stress responses (Osakabe et al. 2016), and growth characteristics (Abdullah, Kamaruddin & Harun 2018). Plant mutation induced by gamma radiation is a well-established plant breeding and genetic research technique. Gamma rays, a form of ionising radiation, can lead to chromosomal alterations like inversions and deletions, resulting in plant mutations (Shirley, Hanley & Goodman 1992). Gamma radiation-induced mutations can be leveraged for genetic improvement in various plant species, especially for arrowroot plant.

MORPHOLOGICAL CHARACTERIZATION OF M3 MUTANTS

Mutation treatment with gamma ray radiation on five (5) mutants of the fourth-generation arrowroot (M3) was only significantly different from the criteria for plant height. However, there was no significant difference in the number of tillers, length, and width of the leaves. This result presented in Table 2. Table 3 presents effect of accession on plant height, number of tillers, leaf width and leaf length of several plant accessions. The fourth generation MN-1 accession has the highest plant height compared to the others. Meanwhile, Cikondang has the lowest plant height. The interaction between accessions and gamma-ray radiation treatment only occurred on plant height criteria. Sudrajat et al. (2023), stated that plant height positively correlates with tuber bioassay and starch content. Thus, as the height of arrowroot plants increases, the yield of tubers and starch also increases. Meanwhile, other plant morphology criteria had no interaction between accessions and gamma-ray radiation treatment. Table 4 shows that 30-gray radiation treatment on the 25 Pandeglang accession was an accession with higher growth compared to other radiation dose. Meanwhile, the Cikondang accession with the 40-gray treatment was the shortest plant accession among the other accessions. This result is the same as previously done by Deswina, Prihastuti and Saputra (2019), where M2 Cikondang with 40 gray treatment is the shortest plant compared to other treatments.

The results of this study indicate that mutation treatment with gamma ray radiation can influence plant growth. Observations of varying plant heights indicate genetic variation in plant responses to gamma-ray radiation treatment. The limited influence of treatment on other plant morphological criteria, such as the number of tillers, leaf length, and leaf width, shows the complexity of plant responses to mutations. According to Jan, Parween and Siddiqi (2012), the mechanism of radiation affecting plant growth and development is still unknown, and the available data is still controversial. Research by Side et al. (2023) highlighted that increasing the radiation dose above a certain threshold decreased sugarcane stalk height. Similarly, Ghasemi-Soloklui, Kordrostami and Karimi (2023) found that higher gamma doses negatively affected vine growth in 'Yaghouti grape plants, reducing plant height, root number, leaf area, and biomass. In contrast, Respati, Umami and Hanim (2018) observed that a 200 Gy gamma radiation dose resulted in the highest number of leaves and tillers in *Brachiaria brizantha* cv. MG5. Moreover, the study demonstrated changes in quantitative traits such as seed number and weight in soybeans due to gamma radiation. Additionally, the research by Guseva, Smolina and Torshin (2022) showed that a dose of 6 Gy inhibited the growth and development of lettuce plants, leading to decreased yield and nutrient uptake. These findings suggest that the effect of radiation dose on plant height, number of tillers, leaf width, and leaf length is dose-dependent and varies across plant species.

Moderate doses of gamma radiation may enhance specific growth parameters; excessive doses can harm plant development. Therefore, optimising radiation doses is crucial to promoting desired morphological traits in plants and maximising agricultural productivity. While low doses may promote plant growth, high doses can have adverse effects, underscoring the importance of precise dosing strategies in radiation-based plant breeding programs. Low radiation doses stimulate cell division, growth, and plant development (Jan, Parween & Siddiqi 2012). Low levels of gamma rays can also induce growth-stimulating signals by increasing the antioxidant capacity of cells or altering hormonal signals in plants (Ali et al. 2015).

The effect of mutation does not always increase plant growth but also causes physiological disturbances due

to the given mutagen (Saragih, Aisyah & Sobir 2019). High doses induce higher damage than low doses because they can inhibit photosynthesis by inhibiting chlorophyll biosynthesis and degradation (Choi et al. 2021). However, administering gamma-ray radiation can cause random mutations that result in faster or slower growth due to physiological damage that occurs during cell development metabolism (Anshori, Aisyah & Darusman 2014). In addition, the cytokinin hormone plays a crucial role in plant growth, especially cell division. Gamma-ray radiation reduces growth regulators such as cytokines by breaking them down or not synthesising them, thereby increasing plant sensitivity (Ali et al. 2015).

CHLOROPHYLL AND CARATENOID CONTENT

Chlorophyll a and chlorophyll b are essential pigments in plants that play crucial roles in photosynthesis and plant health. Chlorophyll a is the primary pigment that captures light energy during photosynthesis, absorbing wavelengths in the violet-blue and reddish-orange-red regions. On the other hand, chlorophyll b acts as an accessory pigment, absorbing light in the green-yellow-orange wavelengths that chlorophyll a does not absorb Grema, Ismail and Muhammad (2022). These two types of chlorophyll work together to maximise light absorption for photosynthetic processes in plants.

The analysis variation of plant pigment content for various arrowroot groups are presented in Table 5. The content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids was not significantly different between treatments, and there was also no interaction between accessions and the dose of gamma radiation. This result shows that the gamma-ray radiation dose treatment did not significantly affect the content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the M3 mutant accession. Although there were no significant differences between treatments in the contents of chlorophyll a and b, there were differences in the contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids between various accessions and doses. The mean of plant pigment content for various groups are presented in Table 6. The highest chlorophyll a was found in the Cikondang 40 gray (4.06), as well as the content of chlorophyll b (2.08), total chlorophyll (6.14), and carotenoids (0.81). Meanwhile, the lowest chlorophyll b content was found in Cikondang 10 gray (1.45), the lowest total chlorophyll content was found in Taman Sari 40 gray (4.38), and the lowest carotenoid content was found in Taman Sari 10 gray (0.41).

Total chlorophyll content in plants is a combination of chlorophyll a and chlorophyll b. The ratio of chlorophyll a to chlorophyll b can vary depending on factors such as leaf age, physiological stress, and genetic mutations

TABLE 2. Analysis of variation in plant height, number of tillers, leaf width and leaf length of arrowroot plants

Source	DF	Mean square			
		Plant height (cm)	Number of tillers	Leaf width (cm)	Leaf length (cm)
Block	2	112.97	46.02*	3.85	4.98
Accession	4	411.37*	36.44	1.31	37.78
Dosage	5	159.74	27.16	4.15	11.85
Accession x Dosage	20	248.46*	161.29	14.00	200.85
Error	58	7800.89	335.98	35.76	332.56
Total	89				
CV		9.27	19.7	8.86	9.37

*significant different on α 0.05

TABLE 3. Effect of accession on plant height, number of tillers, leaf width and leaf length of several plant accessions

Accession (M3)	Plant height (cm)		Number of tillers	Leaf width (cm)	Leaf length (cm)	
Pulosari	126.83	a	12.72	8.78	25.36	ab
25 Pandeglang	129.03	a	10.94	8.77	25.00	b
Cikondang	117.97	b	11.94	8.77	26.79	a
Taman Sari	122.42	ab	12.44	9.06	25.07	ab
MN-1	128.97	a	12.50	8.95	25.58	ab

TABLE 4. Effect of radiation dose on plant height, number of tillers, leaf width and leaf length of several plant accessions

Accession	Dosage	Plant height (cm)	Number of tillers	Leaf width (cm)	Leaf length (cm)
Pulosari	0	126.67abcde	12.00abc	9.09ab	27.50abcd
	10	136.00abc	13.00abc	8.59ab	24.20abcde
	20	121.33abcde	9.67bc	7.96ab	24.69abcde
	30	124.67abcde	12.00abc	9.41ab	27.97abc
	40	119.67bcde	13.67abc	8.54ab	22.96de
	50	132.67abc	16.00a	9.07ab	24.82abcde
25 Pandeglang	0	135.33abc	10.67bc	8.86ab	23.30cde
	10	127.33abcde	12.33abc	8.89ab	25.54abcde
	20	128.00abcde	9.00c	9.50a	28.27ab
	30	143a	10.00bc	8.40ab	22.34e
	40	114.17cde	12.00abc	8.11ab	24.55abcde
	50	126.33abcde	11.67abc	8.83ab	26.02abcde
Cikondang	0	117.00bcde	10.00bc	8.49ab	25.15abcde
	10	113.67cde	11.00bc	9.29ab	27.23abcd
	20	118.83bcde	13.33abc	8.67ab	25.33abcde
	30	130.67abcd	13.33abc	8.31ab	26.41abcde
	40	105.00e	12.00abc	8.46ab	28.96a
	50	122.67abcde	12.00abc	9.41ab	27.65abcd
Taman Sari	0	117.33bcde	12.00abc	9.48a	26.13abcde
	10	109.17de	13.67abc	8.99ab	26.81abcde
	20	129.33abcd	12.67abc	8.43ab	24.10bcde
	30	127.33abcde	12.33abc	8.72ab	22.86de
	40	123.00abcde	13.00abc	9.22ab	25.44abcde
	50	128.33abcd	11.00bc	9.54a	25.09abcde
MN-1	0	139.67ab	14.33ab	9.22ab	26.24abcde
	10	138.33ab	14.00ab	9.41ab	27.44abcd
	20	118.33bcde	13.00abc	9.26ab	25.09abcde
	30	114.67cde	10.00bc	7.88b	25.59abcde
	40	135.83abc	13.67abc	9.07ab	25.69abcde
	50	127.00abcde	10.00bc	8.84ab	23.44bcde

TABLE 5. Analysis of variation chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in arrowroot M3

Source	DF	Mean square			
		chlorophyll a	chlorophyll b	Total chlorophyll	Carotenoids
Block	2	0.45	0.43	1.48	0.01
Accession	4	0.24	0.04	0.42	0.03
Dosage	5	0.30	0.04	0.49	0.01
Accession x Dosage	20	0.39	0.09	0.80	0.02
Error	58	0.30	0.09	0.67	0.01
Total	89				
CV		16.25	17.68	16.04	18.37

TABLE 6. Average content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in arrowroot M3

Accession	Dosage	chlorophyll a	chlorophyll b	Total chlorophyll	Carotenoids
Pulosari	0	3.018±0.436	1.621±0.079	4.639±0.508	0.535±0.178
	10	3.082±0.801	1.585±0.398	4.667±1.198	0.531±0.063
	20	2.951±0.608	1.475±0.431	4.427±1.027	0.544±0.146
	30	3.731±0.067	1.832±0.204	5.563±0.264	0.665±0.113
	40	3.187±0.254	1.516±0.121	4.702±0.370	0.564±0.066
	50	3.600±0.259	1.874±0.182	5.474±0.438	0.611±0.048
25 Pandeglang	0	3.526±0.382	1.752±0.119	5.278±0.491	0.640±0.071
	10	3.688±0.296	1.858±0.234	5.546±0.529	0.661±0.038
	20	3.612±0.291	1.814±0.080	5.426±0.211	0.699±0.097
	30	3.081±0.804	1.724±0.478	4.805±1.282	0.532±0.097
	40	3.258±0.608	1.637±0.349	4.895±0.936	0.550±0.115
	50	3.811±0.850	1.905±0.506	5.716±1.337	0.588±0.041
Cikondang	0	3.794±0.032	1.843±0.092	5.636±0.124	0.717±0.050
	10	2.689±1.295	1.454±0.734	4.143±2.026	0.534±0.187
	20	3.129±0.406	1.569±0.032	4.699±0.411	0.611±0.082
	30	3.298±0.820	1.796±0.503	5.094±1.312	0.599±0.100
	40	4.062±0.708	2.076±0.481	6.138±1.185	0.806±0.129
	50	2.970±0.419	1.488±0.273	4.458±0.692	0.578±0.048
Taman Sari	0	3.853±0.310	1.982±0.120	5.835±0.429	0.613±0.062
	10	2.851±0.630	1.701±0.549	4.552±0.992	0.411±0.265
	20	3.295±0.536	1.701±0.240	4.996±0.776	0.589±0.079
	30	3.485±0.337	1.806±0.194	5.291±0.485	0.599±0.038
	40	2.880±0.103	1.495±0.065	4.375±0.130	0.525±0.046
	50	3.241±0.113	1.683±0.101	4.923±0.146	0.566±0.031
MN-1	0	3.765±1.011	1.604±0.317	5.369±1.040	0.691±0.258
	10	3.546±0.278	1.791±0.177	5.337±0.418	0.685±0.089
	20	3.440±0.500	1.651±0.230	5.090±0.654	0.639±0.042
	30	3.253±0.619	1.699±0.451	4.952±1.066	0.596±0.078
	40	3.370±0.342	1.860±0.308	5.230±0.626	0.540±0.121
	50	3.507±0.375	1.761±0.300	5.268±0.675	0.617±0.056

(Zhang et al. 2017). Studies have shown that changes in chlorophyll content can impact plant growth and development. For instance, the application of UV-B radiation has been found to increase the content of chlorophyll a, chlorophyll b, and total chlorophyll in growing plants (Perucka, Olszówka & Chilczuk 2013). Carotenoids, including xanthophylls and carotenes, are another group of pigments crucial for plant health and human nutrition. These pigments are responsible for the orange, red, and yellow colors in plants and are essential for photosynthesis and antioxidant functions (Maoka 2020). Carotenoids work alongside chlorophylls to capture light energy and protect plant photosystems from photooxidative damage, especially under conditions of increased UV-B radiation (Shen et al. 2017).

Gamma-ray radiation dose treatment did not significantly affect the content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids in the M3 mutant accession. However, some literature mentions that high-dose irradiation inhibits photosynthesis by inhibiting chlorophyll biosynthesis and degradation (Hong et al. 2018; Jan, Parween & Siddiqi 2012). According to a study by Choi et al. (2021), high irradiation doses cause severe physiological damage that cannot be reversed over time. In addition, the efficiency of photosynthesis in rice plants that receive gamma-ray radiation will decrease depending

on the radiation dose. The interplay between plants' chlorophylls, carotenoids, and other pigments is vital for their growth, development, and response to environmental stressors. Monitoring chlorophyll levels in plants can provide insights into their physiological status and response to changing conditions. Understanding the dynamics of these pigments in plants is crucial for optimising plant growth, enhancing crop productivity, and ensuring plant health in various environmental settings.

ANALYSIS OF ARROWROOT GENETICS

Molecular marker analysis using the RAPD is a technique that allows one to detect multiple loci scattered throughout the genome. This technique uses short, random primers consisting of only ten nucleotides. The RAPD marker is suitable for detecting DNA polymorphisms/genetic divergences induced by gamma rays (Dhakshanamoorthy, Selvaraj & Chidambaram 2015). The RAPD technique is relatively simple and easy to use in diversity studies (Chatterjee & Raval 2019). In addition, this technique does not require target DNA sequence information (Panigrahi, Velraj & Rao 2019).

Twenty-two RAPD primers were used in this research, but only 11 produced clear and polymorphic DNA bands to be used for the subsequent analysis stage. Primary selection refers to several aspects, namely the resulting polymorphic band that has good clarity, good reproducibility, stable amplification results, and is easy to read (Gusmiaty et al. 2016). The image of polymorphism bands in mutant arrowroot plants using several RAPD primers is shown in Figure 1. The percentage of polymorphism and PIC of mutant arrowroot plants is shown in Table 7. Several primers with a high polymorphic level are OPA 06, OPW 16, OPAB 03, and OPZ 03 because they can produce polymorphic DNA bands at 100%. Although OPA 06 produced 100% polymorphism, primers OPW 16, OPAB 03, and OPZ 03 were better in the RAPD study than OPA 06 because they produced one allele.

The PIC value of each RAPD primer is determined by the number of alleles and their frequency distribution in a population and is used to assess the level of informativeness (Dwivedi et al. 2018). Therefore, in this study, the PIC of each primer was calculated from 30 populations (5 accessions with six irradiation treatments). PIC values have been used to evaluate genetic variation in

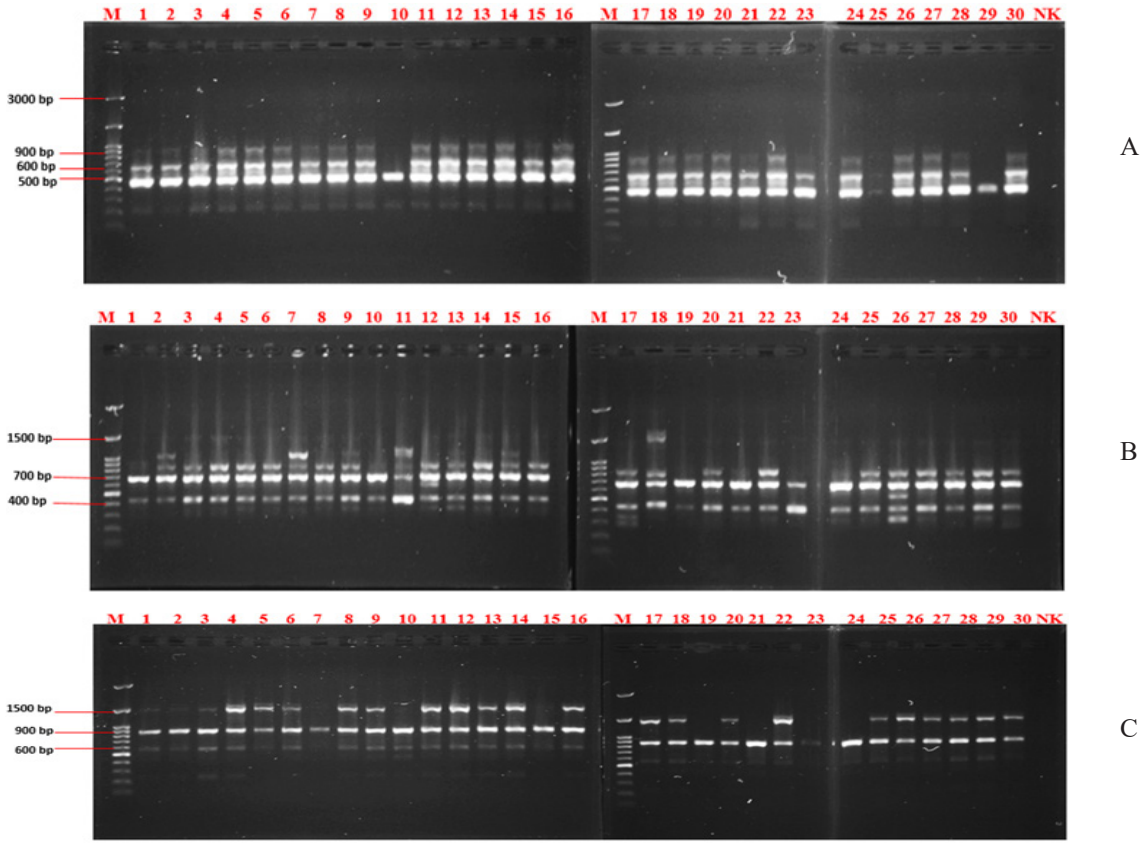

FIGURE 1. Visualization of RAPD results on 30 arrowroot mutant accession numbers using OPAB 03(A), OPD 08 (B), OPAM 03 (C) primers; M:ladder DNA 1Kb

TABLE 7. Percentage of DNA and PIC polymorphic bands from selected primers

Primer	Sekuence	Target	MB	TB	TB	%Polymorfish	PIC
OPD 08	5'-GGGTAACGCC-3'	400-1500 bp	2	7	9	77.78	0.18
OPW 05	5'-GGTCCCTGAC-3'	500-800 bp	2	4	6	66.67	0.08
OPS 19	5'-CTCTCCGCCA-3'	100-700 bp	2	3	5	60	0.17
OPG 13	5'-TGGCGCACAC-3'	300-1500 bp	3	6	9	66.67	0.19
OPA 06	5'-GAGTCAGCAG -3'	800 bp	0	1	1	100	0.06
OPW 16	5'-CTTCCCTGTG -3'	300-1500 bp	0	9	9	100	0.25
OPAB 03	5'-CCGATATCCC-3'	400-900 bp	0	3	3	100	0.19
OPAM 03	5'-GGCGGATAAG-3'	650-3000 bp	1	2	3	66.67	0.2
OPG 18	5'-CAGCCTACCA-3'	300-1000 bp	3	3	6	50	0.14
OPZ 03	5'-AGACGTCCAC-3'	400-1500 bp	0	8	8	100	0.19
OPH 03	5'-GGCTATGTG -3'	200-1000 bp	2	7	9	77.78	0.2
Total			15	53	68	866	1.85
Avarage			1.36	4.82	6.18	79	0.17

MB = Monomorphic Bands, PB = Polymorphic Bands, TB = Total Bands

many studies using RAPD markers (Dhakshanamoorthy, Selvaraj & Chidambaram 2015; Tilwari & Sharma 2021), but PIC is also used in other molecular markers. Based on the PIC information, the primers used have a low category of information. The mean PIC of the 11 RAPD primers was 0.17, with PIC values ranging from 0.06 to 0.25. Primers OPA 06 recorded the lowest PIC and OPW 16 recorded the highest PIC.

Although some primers have a high degree of polymorphism, the PIC values of these primers are low. It could be because the PCR reaction strongly influenced the product of the RAPD primer amplification that was carried out. The PCR parameters that influence RAPD results include DNA concentration, $MgCl_2$ concentration, dNTP concentration, the type and concentration of Taq polymerase, initial denaturation time, temperature, annealing time, reaction volume, and the number of cycles (Martida & Pharmawati 2016; Padmalatha & Prasad 2006). The DNA template must be appropriately purified because contaminated samples can inhibit the PCR reaction (Chatterjee & Raval 2019). In addition, primers that do not match the DNA sequence can prevent the product from being amplified because there is no complementary match between DNA and the primers used (Carsono et al. 2014). The results of primer amplification of OPAM 03, OPD 08, OPF 07, and OPS 19 showed the highest number of DNA band polymorphisms compared to the other seven primers. Analysis with NTSys software used 11 primers as a reference to obtain binary data.

Dendrogram of genetic similarity between 30 mutant arrowroot accession present in Figure 2. Based on the genetic similarity dendogram, the genetic similarity index of the arrowroot mutant is still relatively high, ranging from 0.69 to 0.99. This means that the genetic similarity between the arrowroot mutant is between 69% and 99%. Arrowroot mutant plants can be divided into seven major clusters at a similarity index of 0.84 or a genetic similarity of 84%. Cluster 1 consists of five accessions, namely Pulosari_0 gray, Pulosari_10 gray, Pulosari_20 gray, 25 Pandeglang_0 gray and Cikondang_20 gray. The second cluster only consists of Taman sari_50 gray. Cluster 3 consists of eighteen accessions, namely Pulosari_30 gray, Pulosari_40 gray, Pulosari_50 gray, Cikondang_0 gray, Cikondang_10 gray, Cikondang_30 gray, Cikondang_50 gray, 25 Pandeglang_10 gray, 25 Pandeglang_20 gray, 25 Pandeglang_50 gray, MN-1_0 gray, MN-1 _10 gray , MN-1 _20 gray, MN-1 _30 gray, MN-1 _40 gray, MN-1 _50 gray, Taman sari_10 gray, and Taman sari_30 gray. Cluster 4 consists of two accessions, namely 25 Pandeglang_40 gray and Cikondang 40 gray. Group 5 only consists of 25 Pandeglang_30 gray. Cluster 6 consists of two accessions: Taman sari_0 gray and Taman sari_20 gray. Meanwhile, Cluster 7 only consists of Taman sari_50 gray.

Research conducted by Paradisa, Deswina and Mulyaningsih (2016) on thirty-two accessions of arrowroot plants (before radiation) showed that the germplasm collection was divided into 4 groups and exhibited a genetic diversity of 94%. This indicates that the genetic diversity among these accessions was relatively low. From these 32 accessions, five were selected and then irradiated with various doses of radiation. After mutation, the genetic diversity of the mutants was reanalyzed using RAPD. Using a similarity index of 0.84 or genetic similarity of 84%, mutant arrowroot (M3) can be divided into 7 clusters, the mutants were divided into 7 groups. The lower similarity index means there was an increase in genetic variation among the plants due to the mutation process. This indicates that after mutation, the genetic diversity of

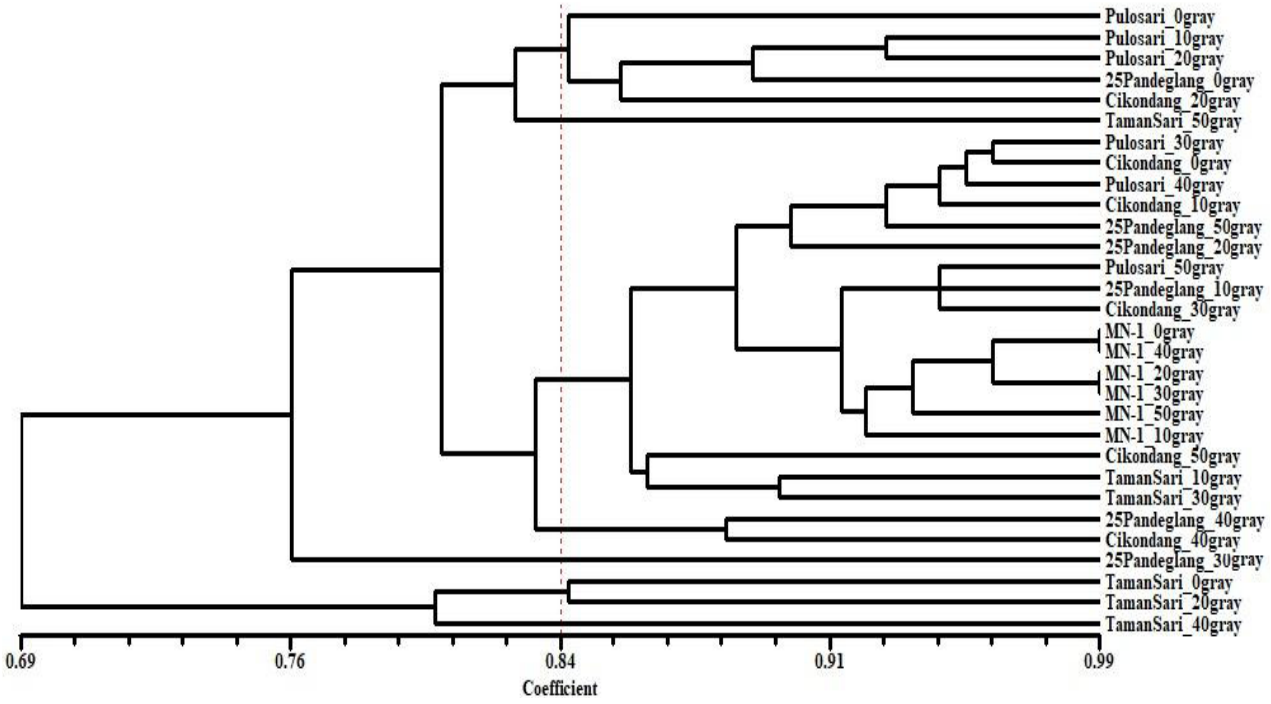

FIGURE 2. Dendrogram of genetic similarity among arrowroot mutants (*Maranta arundinacea*) based on 11 RAPD primers

arrowroot plants had increased, and new groups had formed based on more significant DNA differences. Taman Sari with a radiation dose of 40 gray is thought to have changed genetic composition, this is evidenced by the accession of Taman Sari with a dose of 0 gray and 20 gray as a control in different groups. In the third accession cluster, several accessions were included in the clustering, but several accessions with a dose of 0 gray or as a control were also present in that group. It is suspected that some accessions included in Cluster 3 still have similarities with non-mutant arrowroot tubers.

CONCLUSION

Based on the research conducted, it is known that the treatment of gamma ray radiation is only significantly different to the character of plant height. Radiation had no radiation effect on plant pigment content in M3 mutant arrowroot leaves. The M3 mutant have the genetic similarity index ranging from 0.69 to 0.99. Based on genetic similarity of 84%, mutant arrowroot (M3) can be divided into 7 clusters.

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