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Production Improvement of M1 Generation Garut (*Maranta arundinacea*) Rhizomes through Gamma Irradiation Mutation

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Abstract. Garut (*Maranta arundinacea* L.) is a tuber containing flour with a low Glycemic Index (IG) suitable for diabetics and other degenerative diseases. It needs to be developed to reduce the dependence on imports or to substitute wheat flour. The objective of the study was to obtain superior arrowroot seedlings and observe the effect of gamma-ray radiation on the density and position of stomata on arrowroot leaves. In this research, the irradiation of five arrowroot accessions of second-generation (MV1) with Gamma-rays (0, 10, 20, 30, 40, and 50 Gray) was carried out to obtain mutants with superior character so that they could be used as parent plants for arrowroot flour production. The treatments administered were arrowroot accessions (V), gamma-ray irradiation dose (R), and their interaction. The qualitative and quantitative characters on plant characteristics, tuber production, and arrowroot leaves' stomata were observed. The research results showed that increased production is primarily for 25-Pandeglang accession (808.33 grams) and 10 Gray (800.00 grams) of Gamma-ray irradiation treatment. It is expected that this accession can be released as a new variety candidate after subsequent selection and evaluation in a further generation. Moreover, the dose of gamma-ray irradiation is inversely proportional to the number of stomata, which will increase the photosynthesis, thus increasing the number of tubers produced.

Key words: arrowroot, diversity, gamma-ray irradiation, garut rhizome

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INTRODUCTION

Arrowroot (*Maranta arundinacea* L.), locally known as garut, is a tuber plant belonging to the Marantaceae family. This plant originated from the Central American region, which has now spread to the tropics. The main product of arrowroot tubers is flour extracted from the tubers. Arrowroot rhizomes starch is used as food in various forms such as puddings, jelly, cakes, biscuits, etc. (Lim, 2012) and alternative thickening agents in microbial culture (Marteen et al., 2012). One of the advantages of arrowroot flour is its low glycemic index (IG), making it suitable for diabetic consumption, same as taro (Colocasia spp.) (Oktavianingsih et al., 2017). Arrowroot flour levels were 75.48% (Elvandari et al., 2020), while the IG of oyek and tiwul arrowroot were 40 and 41, respectively (Hasan et al., 2012). Oyek and tiwul is a traditional Indonesian Javanese food made from cassava and is a staple substitute for rice. The previous research showed that the arrowroot flour characteristics were influenced by the cultivation factors, including geographical location (Sholichah et al., 2019).

Wheat flour substitution with other sources of flour can reduce the need for wheat flour and strengthen the food security system (Cahyana et al., 2020). Although several researchers have carried out arrowroot research, intensive planting has not yet been done on a large scale. One reason is the provision of quality seeds in production and starch levels. Arrowroot is usually propagated vegetatively to produce a narrow genetic diversity (Sukamto et al., 2016). The provision of superior seeds on a large scale is crucial for wheat flour substitution. An analysis of diversity conducted by Suhartini (2016) showed that the diversity of arrowroot plants is narrow, with a 7-25.5% diversity coefficient. It is necessary to expand the genetic diversity of arrowroot to obtain superior arrowroot plants with high productivity and starch content. Plants propagated vegetatively are generally heterozygous, and their genetic diversity can be expanded by irradiation (Asha et al., 2015). The objective of the study was to obtain superior arrowroot seedlings and observe the effect of gamma-ray irradiation on the density and position of stomata on arrowroot leaves. Stomata play an important role in the passage of carbon dioxide, water vapor, and oxygen from the leaves (Sreelakshmi et al., 2014). Therefore, the large number of stomata on leaves can affect photosynthesis and tuber production in plants.

METHODS

The study was conducted at the Laboratory of Agronomy and Germplasm Garden (KPN) LIPI Cibinong Science Center (CSC), Bogor Regency, West Java (6 ° 29'48" Southern Latitude and 106°51'17" East Longitude). Arrowroot material used in this study was taken from Germplasm Garden of Research Centre for Biotechnology-LIPI (Table 1).

Table 1. Arrowroot plant collection at KPN, CSC-BG

Code	Accession	Origin
A	Pulosari	West Java
B	25 Pandeglang	West Java
C	Cikondang	West Java
D	Tamansari	DI Yogyakarta
E	MN-1	West Java

The second-generation (M1) arrowroot tuber yields were selected, sun-dried, and then sown in cocopeat media on 20 x 30 cm polybags. The seedlings of 2-5 cm diameter, 4-7 cm long with 2-4 buds were used (Fig 1A). Bulbs (± 1-2 cm) were immersed in the seedling media. Observations were carried out until shoots emerged from the media surface (Figure 1B). Shoots on the surface were then counted and measured for their length. After two months, the seedlings were transferred to mixed soil media, manure, and rice husk (2: 1: 1) in 50 x 50 cm polybags.

Figure 1. Second generation arrowroot tuber (A) and arrowroot bud growth (B)

Plant seeds of ± 5 cm in height were transferred to the growing media in a polybag. Fertilization was done at the time of planting, after 3-4 months, and before the plants bloomed or formed tubers (6-7 months). Seedling maintenance was done by weeding and keeping adequate media in a polybag so that the tuber formation in the soil was not disturbed. Watering and controlling of pests and diseases were adjusted to conditions.

Gamma-ray irradiation

A similar size of 9 months arrowroot tubers (± 2 cm) was wrapped in aluminum foil and sent to the Central Laboratory of Isotope and Irradiation Applications, the National Nuclear Energy Agency (BATAN) for irradiation for ± 5 minutes at 10-50 Gray doses at 10 Gray intervals.

Morphological observations

Observations were made qualitatively and quantitatively. Qualitative variables were: leaf color characteristics, leaf midrib color, leaf stalk color, while the quantitative characters were: plant height, number of productive tillers and number of segmented tillers, leaf length, and leaf width. Observations were made six months after planting. The other parameters are tuber characters (tuber length, tuber diameter, total tuber weight, wet tuber weight, and dry tuber weight) observed after harvest (9 months). The data analysis used descriptive statistical methods such as averages, standard deviations, coefficient of diversity, and correlations between quantitative characters. Variance testing was conducted, followed by Duncan's multiple range test at 5% significance level.

Stomata observation

The observation of stomata was only carried out on accession Taman sari (D). The number of stomata was calculated at the end of the observation at noon. The leaves used were the first, second, and third leaves, calculated based on the first leaf that had completely bloomed on the parent plant. Observations were made using a Nikon light microscope (40 x magnification). The arrowroot leaves to be observed were cut. The leaf stalk was inserted into a bottle containing water prior to the observation of stomata. The leaf's surface was cleaned with 70% alcohol, 2.5 cm of scotch tape was pasted on the leaf, rubbed evenly with the nail polish, and let dry for about one hour, 2.5 cm of scotch tape then pasted on the nail polish-covered leaf. The nail polish was removed from the sticky tape, and the leaf was placed on the glass preparation.

The percentage of stomatal density and damage calculation:

$$Stomatal\ Density = \frac{Number\ of\ stomata}{Field\ of\ view\ width}$$

Field of view width for magnification 400 x = ¼ π d2 = ¼ x 3.14 x (0.5) 2 = 0.19625 mm2. The leaf width of each experimental unit was taken from 3 leaves representing each treatment. Stomatal damage, adopted from Rosmaina et al. (2019), was calculated using the following formula:

$$\frac{Damaged\ Stomata}{Observed\ stomata}\ x\ 100\%$$

Experimental design and data analysis

The study used a Randomized Block Design (RBD) Factorial with three replications. The first factor was five accessions of arrowroot plants (Table 1.), the second factor was the dose of gamma-ray irradiation, namely: 0 (control), 10 Gray, 20 Gray, 30 Gray, 40 Gray, and 50 Gray at 10 Gray intervals.

RESULTS AND DISCUSSION

The average value of arrowroot growth at the age of 6 months is displayed in Table 2. The results showed that all accessions did not show a significant difference in growth in the arrowroot plants aged six months, except for the number of productive tillers. The highest number of tillers (10.83) was obtained from accession Pulosari (A), while the least amount was from accession Tamansari (D). The gamma-ray irradiation dose significantly affected the growth parameters (Figure 2). According to Aisyah & Darusman (2014), gamma-ray irradiation can change the phenotypic characters of plants, such as the shape of leaves and stems. Table 2 shows that 40 Gray and 50 Gray irradiation doses significantly affected plant height parameters, where the plants were shorter than the plant height in the 30 Gray, 20 Gray, 10 Gray, and control treatment. Gamma-ray irradiation treatment with high doses can cause physiological stunted plant growth. However, it is hoped that this mutation with gamma-rays will produce superior plant candidates (Saragih et al., 2020). A study conducted by Aisyah & Darusman (2014) on turmeric (*Curcuma domestica*) showed a very close relationship between increasing the irradiation dose and decreasing plant growth, except for the 3rd leaf stalk length character, which was closely related. In contrast, the interaction between arrowroot plants' accessions with irradiation doses did not produce a significant difference in all observed growth parameters. However, the interaction between accession and gamma-ray irradiation can be made in the next generation.

Table 2. The arrowroot plants growth observation for 6 months

Treatments	Plant Height (cm)	Number of productive tillers	Number of segmented tillers	Leaf length (cm)	Leaf width (cm)
Accessions					
Pulosari (A)	111.69	10.83b	3.28	28.98	10.16
25 Pandeglang (B)	105.06	9.22ab	3.39	27.63	9.71
Cikondang (C)	104.92	9.78ab	3.22	28.86	10.01
Tamansari (D)	106.81	8.50a	3.39	28.85	10.46
MN-1 (E)	108.67	9.89ab	3.39	27.65	10.09
Gamma-ray irradiation (Gray)					
0	120.30c	10.87b	4.47b	29.89bc	10.71c
10	120.53c	9.33ab	3.13a	30.56c	10.61bc
20	114.10c	8.80a	2.80a	29.43bc	10.08abc
30	103.77b	9.13ab	3.20a	27.38ab	9.56a
40	94.90a	9.73ab	3.20a	26.02a	9.83abc
50	90.97a	10.00ab	3.20a	27.08ab	9.73ab

Note: The RBD Factorial was analyzed using IBM SPSS Statistics 23. Further analysis was done using the Duncan Multiple Range Test (DMRT). Mean values within a column followed by the different letters are significantly different ($p<0.05$).

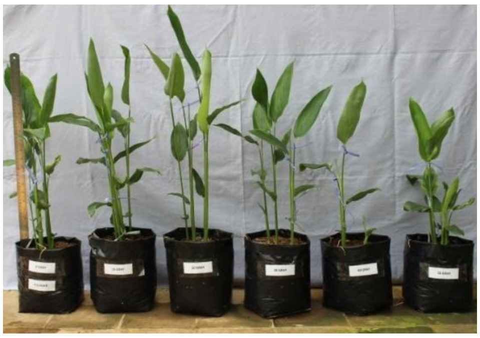

Figure 2. The growth appearance of arrowroot accessions from Pandeglang (A) irradiated with 10 Gray, 20 Gray, 30 Gray, 40 Gray and 50 Gray (from the left to the right side)

Qualitative observation

Observation results of leaf, leaf midrib and leaf stalk color are shown in Table 3.

Table 3. Leaf colors of four arrowroot accessions after being irradiated with gamma-rays

Accessions	Irradiation dose (Gray)	Leaf color	Leaf midrib color	Leaf stalk color
Pulosari (A)	0	H	HPUTP	HM-HSU-U
	10	H	HPUTP	HM-HSU-U
	20	H	HPUTP	HM-HSU-U
	30	H	HPUTP	HM-HSU-U
	40	H	HPUTP	HM-HSU-U
	50	H	HPUTP	HM-HSU-U
25 Pandeglang (B)	0	H	HPUTP	HM-HSU-U
	10	H	HPUTP	HM-HSU-U
	20	H	HPUTP	HM-HSU-U
	30	H	HPUTP	HM-HSU-U
	40	HB	HPUTP	HM-HSU-U
	50	H	HPUTP	HM-HSU-U
Cikondang (C)	0	H	HPUTP	HM-HSU-U
	10	H	HPUTP	HM-HSU-U
	20	H	HPUTP	HM-HSU-U
	30	H	HPUTP	HM-HSU-U
	40	H	HPUTP	HM-HSU-U
	50	H	HPUTP	HM-HSU-U
Tamansari (D)	0	H	HPUTP	HM-HSU-U
	10	H	HPUTP	HM-HSU-U
	20	H	HPUTP	HM-HSU-U
	30	H	HPUTP	HM-HSU-U
	40	H	HPUTP	HM-HSU-U
	50	H	HPUTP	HM-HSU-U
MN-1 (E)	0	H	HPUTP	HM-HSU-U
	10	H	HPUTP	HM-HSU-U
	20	H	HPUTP	HM-HSU-U
	30	H	HPUTP	HM-HSU-U
	40	H	HPUTP	HM-HSU-U
	50	H	HPUTP	HM-HSU-U

Note: H = Green; HB=Striped; HTTP = Green purple edge white spot; HM = light green; HSU = Greenish purple; U = Purple

Table 3 shows that the irradiation dose of 0-50 Gray does not affect the color of the leaves, midribs, and stems except the color of the leaves of accession 25 Pandeglang (B) after being irradiated with Gamma-rays 40 Gray shows stripes of chimera/variegata. However, stripes occurred even on arrowroots leaves that were not irradiated (Suhartini, 2016). According to Kaur et al. (2017) different doses of gamma-ray irradiation can have a distinctive effect on the biochemistry, physiology, and morphology of plants. Furthermore, Hidayati et al. (2012) found that 40 Gray-gamma irradiation treatment resulted in the higher Drought Tolerance Index of arrowroot.

Production

Post-harvest observations (9 months after planting) showed that the accession of arrowroot plants was significantly different in tuber diameter, tuber wet weight, and tuber dry weight. In contrast, the dose of gamma-ray irradiation produced significant differences in tuber length, total tuber weight, and tuber dry weight. The wet weight production of tubers in accession 25 Pandeglang (B) was the highest (808.33 gram) and irradiation treatment with a dose of 10 Gray. The interaction between plant accessions and gamma-ray irradiation doses did not produce significant growth differences (Table 4). There is still a need for further selection and evaluation of the selected accessions in the next generation. Research conducted by Suhartini (2016) at Cikeumeuh showed that clump tubers' weight was positively correlated with plant height, the number of leaves, length, tuber circumference, and negatively correlated with leaf length, leaf width, and leaf stalk length.

Table 4. Observation of post-harvest arrowroot (9 months) irradiated with gamma-rays

Treatments	Tuber length (cm)	Tuber diameter	Total tuber weight (gram)	Tuber wet weight (gram)	Tuber dry weight (gram)
Accessions					
Pulosari (A)	20.25	15.73ab	3661.11	663.89ab	335.17a
25 Pandeglang (B)	20.49	17.08b	3388.89	808.33b	468.56b
Cikondang(C)	21.16	14.75a	3700.00	702.78ab	389.54ab
Tamansari(D)	21.26	15.99ab	3275.00	602.78a	328.00a
MN-1(E)	19.38	16.16ab	3605.56	719.44ab	378.11ab
Gamma-Ray Irradiation (Gray)					
0	21.85bc	15.38	3286.67a	670.00	407.03bc
10	22.17c	17.21	3263.33a	800.00	492.30c
20	23.36c	16.44	2926.67a	753.33	469.67c
30	19.48ab	15.29	3510.00a	616.67	314.73ab
40	18.87a	16.06	3603.33a	620.00	276.33a
50	17.33a	15.27	4566.67b	736.67	319.18ab

Note: The RBD Factorial was analyzed using IBM SPSS Statistics 23. Further analysis was done using the Duncan Multiple Range Test (DMRT). Mean values within a column followed by the different letters are significantly different ($p<0.05$)

Table 5 shows that the arrowroot leaves have many stomata (101-200). In general, an increase in the dose of gamma-ray irradiation would significantly reduce the arrowroot leaves' stomatal density. The highest number of stomata was obtained from the 10 Gray gamma-ray treatments (141.37), while the lowest (107.67) was obtained from the 50 Gray irradiation dose. Stomata are one of the most important physiological apparatuses in higher plants that control gas exchange and water transpiration, nutrient uptake, plant growth, development, etc. (He et al., 2019). In contrast, the stomatal position in the arrowroot leaves did not show a significant difference in the density of the stomata, as does after the interaction with the gamma-ray irradiation dose. Likewise, the morphology of the stomata at the tips, middle, and base of the leaf does not look different. The appearance of 40x magnification of arrowroot leaf stomata is shown in Figure 3. Rohandi et al. (2017) have shown that the stomatal density of arrowroot leaves was positively correlated with the rhizome's moisture content.

In contrast, in soybean plants (Glycine max) fertilized with Nano-silica and Plant Growth Promoting Rhizobacteria (PGPR), a positive correlation occurred between stomatal openings and soybean yields (Suryanti & Umami, 2020). Transpiration rates are quicker in the leaf with more stomata (Klarisya & Daningsih, 2021). The frequency and position of stomata are characteristics of organs and species that have certain characters. It is also influenced by environmental factors (Garvita & Wawangningrum 2020).

In general, mutation treatment with Gamma-ray irradiation in arrowroot plants aims to obtain mutants with better morphological properties than before and high productivity. Accessions of 25 Pandeglang and Cikondang can be selected as accession candidates with irradiation doses of 10 and 20 Gray. With the right dose of Gamma-ray irradiation treatment, plants with superior characteristics such as high yields, shorter harvest life, and other desirable properties will be selected and evaluated for obtained superior candidates (Rohandi et al., 2017).

Table 5. Stomatal density of Tamansari accession (D)

Treatment	Stomatal density/ Field of view*)
Gamma-Ray Irradiation (Gray)	
0	140.59b
10	141.37b
20	120.93a
30	138.07b
40	113.37a
50	107.67a
Stomata location	
Base	129.31
Middle	124.44
Tip	127.24

Note: - The RBD Factorial is analyzed using IBM SPSS Statistics 23. Further analysis was done using the Duncan Multiple Range Test (DMRT). Mean values within the column followed by the different letters are significantly different ($p<0.05$). *) Area per field of view = 0.19625 mm^2.

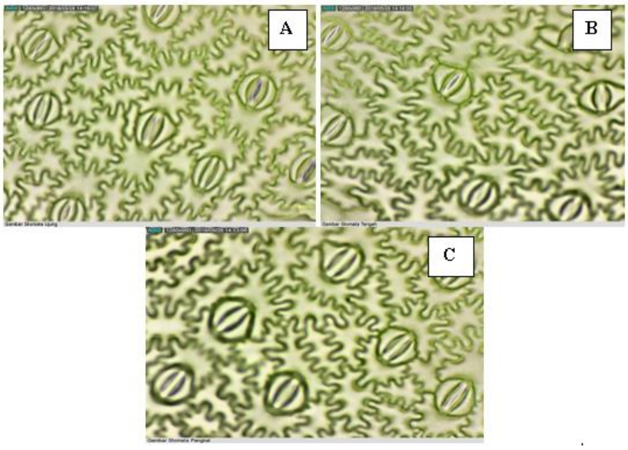

Figure 3. Arrowroot leaf stomata of 40x magnification A. Tip: B. Middle; and C. Base

CONCLUSION

Gamma-ray irradiation treatment significantly affected the morphological characters of arrowroot plants, especially on plant height. At a 50 Gray Gamma-ray irradiation dose, the shortest plant (90.97 cm) was obtained, compared to the control plant height (120.37 cm). The higher the irradiation dose, the lower the plant height growth. On the other hand, its highest tuber produced from accessions of 25 Pandeglang (808.33 gram) and Cikondang (702.78 gram) with irradiation doses of 10 Gray and 20 Gray. After following the selection and evaluation series in the next generation, these two selected accessions can be used as superior candidates.

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