CYTOMORPHOLOGICAL CHARACTERISTIC EVALUATION OF THE THIRD GENERATION OF ARROWROOT PLANT (*Maranta arundinacea* L.) RADIATED BY GAMMA RAY

EVALUASI KARAKTERISTIK CYTO-MORFOLOGI GENERASI KETIGA TANAMAN GARUT (Maranta arundinacea L.) HASIL RADIASI SINAR GAMMA

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ABSTRACT

Arrowroot plants (*Maranta arundinacea* L.) have enormous potential to be developed as the alternative foods. Various kinds of functional food products can be processed from arrowroot tubers, because they have lower calories and IG (*Glycemic Index*) contents compared to the other tubers. The study aimed to determine the characteristics of the phenotype, productivity and anatomy of leaves of arrowroot plants radiated by gamma ray. The study used factorial randomized block design with 2 factors. The first factor was arrowroot accessions (Pulosari, 25 Pandeglang, Cikondang, Tamansari, and MN-1), and the second factor was radiation dose (0, 10, 20, 30, 40, 50 Gray), and those was arranged in three replications. Based on the results of the study, it found that: Pulosari accession without radiation and 30 Gray radiation required the fastest time of 12 days for shoots growth. The highest plant growth (106,88cm) was Cikondang accession with 20 Gray radiation treatment; while the shortest plant (59.61cm) was found in Cikondang accession with 30 Gray radiation dose as well. The number of productive tillers (3.0) was mostly found in Pulosari accession without radiation, conversely, the least (0.72) was found in MN-1 accession with 20 Gray radiation. For the maximum number of out-of-section tillers (1.17) on the 25 Pandeglang accession with 40 Gray radiation. The longest leaf growth (22.64 cm) and the widest (9.08 cm) were found in Cikondang accession without radiation, conversely, the shortest leaf growth (10.17 cm) and smallest (3.34 cm) was found in Cikondang accession radiated by 40 Gray. There were no changes to the number and shape of the stomata between the control plants and the radiated treatment, but there were changes in leaf color on 25 Pandeglang accession with radiation treatment 40 Gray.

Key words*: arrowroot, gamma radiation, alternative food, diversity*

ABSTRAK

Tanaman Garut (*Maranta arundinacea* L.) memiliki potensi besar untuk dikembangkan sebagai pangan alternatif. Berbagai macam produk pangan fungsional dapat diolah dari umbi

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Garut, karena memiliki kandungan IG (*Index Glycemic*) dan kalori lebih rendah dibandingkan umbi-umbi lainnya. Penelitian ini bertujuan untuk mengetahui karakteristik fenotipe, produktivitas dan anatomi daun tanaman Garut yang diberi perlakuan radiasi sinar gamma. Penelitian disusun menggunakan Rancangan Acak Kelompok (RAK) Faktorial dengan 2 faktor, dengan faktor pertama aksesi tanaman (Pulosari, 25 Pandeglang, Cikondang, Tamansari, dan MN-1), dan faktor kedua adalah dosis radiasi (0, 10, 20, 30, 40, 50 Gray), penelitian terdiri dari 3 ulangan. Berdasarkan hasil penelitian diketahui bahwa aksesi Pulosari tanpa radiasi dan radiasi 30 Gray memerlukan waktu keluar tunas paling cepat yaitu 12 hari. Pertumbuhan tanaman paling tinggi (106,88 cm) adalah aksesi Cikondang dengan perlakuan radiasi 20 Gray, sedangkan tanaman paling pendek (59,61 cm) juga terdapat pada aksesi Cikondang dengan dosis radiasi 30 Gray. Jumlah anakan produktif (3,0) paling banyak terdapat pada aksesi Pulosari tanpa radiasi, sebaliknya yang paling sedikit (0,72) terdapat pada aksesi MN-1 radiasi 20 Gray. Untuk jumlah anakan keluar ruas paling banyak (1,17) pada aksesi 25 Pandeglang dengan dosis radiasi 40 Gray. Pertumbuhan daun paling panjang (22,64 cm) dan paling lebar (9,08 cm) selama pengamatan terdapat pada aksesi Cikondang tanpa radiasi. Sebaliknya pertumbuhan daun paling pendek (10,17 cm) dan kecil (3,34 cm) juga terdapat pada aksesi Cikondang dan radiasi 40 Gray. Tidak terdapat perubahan terhadap jumlah dan bentuk stomata antara tanaman kontrol dengan perlakuan radiasi, akan tetapi terdapat perubahan warna daun pada aksesi 25 Pandeglang dengan perlakuan radiasi 40 Gray.

Kata Kunci: *Garut, radiasi sinar gamma, pangan alternatif, keragaman*

INTRODUCTION

Arrowroot (*Maranta arundinacea* L.) is an alternative food source with high economic value and great potential to be developed as a raw material for developing functional-food products, especially for people with diabetes and digestive disorders. Arrowroot plants are herbaceous with a shallow root. Its bulbs are enlarged rhizomes in cylindrical forms; the leaves are elongated in oval-shape with the leaves blade encircling the trunk (Asha *et al*., 2015).

Arrowroot contains starch so it is potentially substitutes wheat flour (Hariyadi, 2010) or other common flours (Djaafar & Rahayu, 2006). Arrowroot contains carbohydrates that can be used for food and industrial raw materials (Ramadhani *et al*., 2017) with highly nutritious; 25-30% carbohydrate and ± 20% starch (Rahman *et al*., 2015). Arrowroot tuber offers health benefits because its glycemic index is as low as 14, lower than flour, rice, potatoes, and cassava, which are around 100, 96, 90 and 54, respectively (Rahman *et al*., 2015). Arrowroot plants boost high economic value and can be found in all parts of Indonesia because its cultivation is relatively easy and does not require particular handling. By intensive cultivation these plants can produce an average of 21 t ha^{-1} (Sarjiman & Djaafar, 2007).

Arrowroot is an introduced plant from Central America and South America that has adapted to the conditions in Indonesia. The plant can live on marginal or empty lands under a tree. Additionally, arrowroot cultivation have been done by farmers on non-irrigated or rainfed dry land. Arrowroot belongs to the family of Marantaceae, Maranta genus, *Maranta arundinacea* L. species, and is grouped to minor tubers. The local names of arrowroot are diverse, in West Java they are called *patat sago, irut, arut,* and *jelarut*; in Madura *selarut* or *larut*; in Gorontalo *labia walanta*; in Ternate *huda sula*, in Halmahera *peda sula*; and in America it's called Arrowroot (Djaafar *et al*., 2010). Arrowroot plants originated from America, specifically at the tropics, and then it spreads to other tropical countries (Shintu *et al*., 2016; Faridah *et al*., 2014).

The genetic diversity of arrowroot germplasm plays an important role in supporting the improvement of its own superior varieties, especially characters related to starch content and high yield (Sarjiman & Djaafar, 2007). In the present, the opportunity to increase the genetic diversity of arrowroot through crossing is still difficult because its vegetative propagation and narrow number of arrowroot species. Induction mutation is an appropriate breeding method, especially for plants that has vegetative breeding (Soeranto, 2003). Induction of mutations with gamma rays is the best technique compared to chemical methods, because it can produce the most mutants (around 75%). In addition, gamma rays are more accurate and the penetration of radiation into cells is homogeneous (Zanzibar & Witjaksono, 2011).

The results showed that the dose of gamma ray radiation in the M4 generation was significantly different in germination time, seed germination energy, and plant height (Gurning *et al*., 2013). The results of Jan *et al*. (2011) research on some arrowroot plants from Malang, Bogor, and Bantul showed no significant differences in their morphological characters. The results of the analysis of the genetic diversity of 19 Indonesian arrowroot accessions carried out through molecular markers of RAPD have shown that they are not genetically different, thus the limited supply of superior arrowroot genotypes from existing germplasm is one of the limiting factors in arrowroot breeding programs (Deswina *et al*., 2019).

The objective of this study was to determine the cytomorphological characters of third generation arrowroot plants radiated by gamma ray. Induction mutations can cause genetic diversity, because genetic material changes at the level of the genome, chromosomes, and DNA or genes (Giono *et al*., 2014). Through mutation breeding a number of arrowroot mutants will be produced that are better than the original germplasm.

MATERIALS AND METHODS

This research was conducted on February 2018 - November 2018, at the Agronomy Lab and Germplasm Gardens (GG) LIPI Cibinong Science Center (CSC), Bogor Regency, West Java Province. The study was arranged by using Randomized Block Design (RBD) Factorial with three replications. Factor I consisted of 5 accessions of arrowroot plants (Table 1); Factor II was the dose of gamma irradiation consisting of: 0 (control), 10 Gray, 20 Gray, 30 Gray, 40 Gray, and 50 Gray.

Table 1. Arrowroot Plant Collection at Germplasm Garden (GG) LIPI Cibinong Science Center, Bogor Regency, West Java Province

Code	Accessions	Origin
a1	Pulosari	Jawa Barat
a2	25 Pandeglang	Jawa Barat
a3	Cikondang	Jawa Barat
a4	Tamansari	DI Yogyakarta
a5	MN-1	Jawa Barat

The second generation of arrowroot mutant (M_2V_1) was germinated in the seedling media consist of coco peat, which dried by the sun. The germinated seed selected from the healthy ones; 2-5 cm in diameter, 4-7 cm in length, with 2-4 buds (Fig 1A). Bulbs were immersed approximately 1-2 cm into the seedling media. Observations were made until the buds appeared to surface (Figure 1B). The planting media used in the polybag; soil, manure, and husks mixed with a ratio of 2:1:1. The size of the polybag was 50cm x 50cm.

A B

Figure 1. The second generation of arrowroot shoots (A) and tubers (B

Particularly, loose soil structure is preferable for tuber growth. Moreover, the harvesting process would also be easier and faster if the soil were loose. The shoots of arrowroot plants that germinated and measured to the 5 cm tall could be transferred to the planting media. Fertilizers was applied in the beginning of cultivation. Afterwards, in 3-4 months when the plant begin to bloom or when tubers were starting to emerge from 6-7 months. Weed control by removing weeds and maintaining adequate media in polybags so that the formation of tubers in the soil will be undisturbed. Weeding, pest and disease control can be adjusted according to needs.

Morphological Observation

Data collected includes qualitative and quantitative observations. The qualitative variables collected to complete the quantitative characters, such as leaf color, leaf mid-vein color and leaf vein color. The quantitative characters variables being observed were; plant height, leaf length and leaf width, number of tillers and number of tillers per clump.

The data were tested using correlation between quantitative characters, analysis of variance followed by Duncan's multiple range test at the 5% level (Gomez and Gomez 1995). Linier model are showed in equation (1):

$$Y_{ijk} = \mu+\alpha_i+\beta_j+(\alpha\beta)_{ij}+\rho_k+\sum_{ijk} \ldots\ldots\ldots\ldots \quad (1)$$

Note:

Y_{ijk}	The value of observations on factor A level I factor B level j and kth group
$(\mu\ \alpha_i\ \beta_j)$	Additive components from the average, the main influences of factors A and the main influence of factor B.
$(\alpha\beta)_{ij}$	Component of interaction of factor A and factor B.
ρ_k	Additive influence from the group and assumed no interact with treatment (additive).
$\sum_{ijk}$	Experiment error.

Observation of Anatomical Stomata

The leaves of the arrowroot plant were cut, put the stem into a bottle of water, clean the surface of the leaf with 70% alcohol, attach 2.5 cm of scotch tape on the leaf by applying a sufficient pressure, coat the leaf with nail polish thoroughly, leave it to dry (± 1 hour). Apply 1.2 cm of scotch tape on the surface of the leaf coated with nail polish, remove the attached nail polish and put it on the preparation glass. The stomata preparation can then be observed under a microscope. Calculate stomata density and damage percentage with the following formula:

$$\text{Stomata Density} = \frac{\text{The number of stomata}}{\text{Field of View Area}} \quad \ldots.(2)$$

Where, broad field of view for enlargement 400 x = ¼ π d2 = ¼ x 3,14 x (0,5)2 = 0,19625 mm^2. Measuring the leaf width of each experimental unit, 3 leaves were chosen to represent each treatment. While stomata damage can be calculated with the following formula, adopted from Lestari (2006):

$$\frac{\text{The amount of damaged Stomata}}{\text{The amount of Stomata being observed}} x\ 100\% \quad \ldots.(3)$$

RESULTS AND DISCUSSION

Morphological Characteristics

1. Growth of the Shoots

Based on the observations of shoots growth, Pulosari accession with the control treatment (a1e0) and a1r3 have shown better shoot growth compared to other treatments, it took only ± 12 days for it to grow. While for Cikondang accession a3r3 and a3r4 treatment would take the longest shoots growth of about ± 47 days. Tubers administered with high doses of irradiation (40 and 50 Gray) have much slower growth rate compared to the others (Table 2). The factors were suspected from external factors. Hidayat *et al.* (2012) stated that the growth of bitter plants became slower due to the irradiation treatment. According to Yuniastuti (2012), arrowroot seeds must go through a shelf life to break dormancy before being used for subsequent planting. Lathifah *et al*. (2017) stated that tubers that are ready for planting are tubers that have germinated around 2 cm. Tubers that have not germinated or are in a period of dormancy are not suitable to be planted because the growth is slower, sometimes they would rot underneath the soil and the tuber yield is very poor. Data on the length of shoot growth showed in Table 2.

Table 2. The combination of accession and radiation treatment on the time of shoot release.

Treatment	Duration (Days)	Notes	Treatment	Duration (Days)	Notes
a1r0	12	-	a3r3	47	-
a1r1	21	-	a3r4	47	-
a1r2	21	-	a3r5	35	-
a1r3	12	-	a4r0	35	Embroidered
a1r4	32	-	a4r1	32	-
a1r5	21	-	a4r2	35	-
a2r0	21	-	a4r3	21	-
a2r1	21	-	a4r4	35	-
a2r2	21	-	a4r5	32	-
a2r3	35	-	a5r0	45	Embroidered
a2r4	32	-	a5r1	21	-
a2r5	35	-	a5r2	35	-
a3r0	14	-	a5r3	35	Embroidered
a3r1	32	-	a5r4	32	Embroidered
a3r2	32	-	a5r5	32	Embroidered

Note: A1 (Pulosari), A2 (25 Pandeglang), A3 (Cikondang), A4 (Tamansari), dan A5 (MN-1). R0 (kontrol), R1 (10 Gy), R2 (20 Gy), R3 (30 Gy), R4 (40 Gy), dan R5 (50 Gy).

2. Plant Height

The plant heights were observed in 4, 5, and 6 months after planting (MAP). Plant height is a growth indicator or a parameter to determine the effect of the environment on treatment. Plant height is the most observable measure of growth (Gusmaini *et al*., 2003). The results of analysis of variance have shown that the accession, radiation, and interactions factors had a significant effect on plant height at 4, 5 and 6 MAP.

Table 3. Interaction between accessions and radiation doses on the height of arrowroot plants aged 4, 5, 6 months after planting.

Interactions	Average (cm)		
	Month 4	Month 5	Month 6
a1r0	57.08 cd	74.48 cd	88.91 cd
a1r1	59.72 cd	78.19 d	96.94 d
a1r2	61.57 d	87.07 de	89.68 cd
a1r3	66.29 d	90.33 e	99.38 d
a1r4	45.40 bc	55.49 b	73.06 b
a1r5	66.70 d	88.32 de	103.78 d
a2r0	64.91 d	92.38 e	100.25 d
a2r1	63.49 d	80.53 de	96.18 cd
a2r2	57.79 cd	86.03 de	102.50 d

Interactions	Average (cm)		
	Month 4	Month 5	Month 6
a2r3	56.68 cd	76.87 cd	101.58 d
a2r4	56.43 cd	72.73 cd	85.88 c
a2r5	51.14 cd	79.48 de	89.15 cd
a3r0	67.87 d	86.91 de	96.63 d
a3r1	54.40 cd	83.55 de	97.31 d
a3r2	65.81 d	92.93 e	106.88 d
a3r3	39.87 bc	67.41 a	88.29 cd
a3r4	20.43 a	41.01 a	59.61 a
a3r5	43.02 bc	69.23 cd	82.00 bc
a4r0	58.38 cd	82.89 de	100.82 d
a4r1	62.08 d	77.25 cd	93.48 cd
a4r2	49.15 cd	73.20 cd	92.92 cd
a4r3	66.84 d	88.81 e	102.39 d
a4r4	39.45 bc	62.83 bc	82.28 bc
a4r5	51.78 cd	81.48 de	95.36 cd
a5r0	44.48 bc	69.73 cd	88.80 cd
a5r1	55.84 cd	83.30 de	88.58 cd
a5r2	34.21 b	63.77 bc	81.99 bc
a5r3	35.74 bc	65.10 bc	89.13 cd
a5r4	43.78 bc	61.28 bc	83.11 bc
a5r5	48.01 c	75.20 cd	91.31 cd

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test.

From Table 3, it was found that the interaction between accessions and radiation doses at 6 MAP of the a3r2 treatment provided the highest results (106.88 cm), while the A3R4 treatment produced the shortest plant height (59.61 cm). a1r0 treatment (control) was not significantly different from a1r2 but was significant from a1r1, a1r3, a1r4 and a1r5. a2r0 treatment was not significantly different from a2r1, a2r2, and a2r3 but was significantly different from a2r4 and a2r5. The a3r0 treatment was not significantly different from the a3r1, a3r2, and a3r3 but was significantly different from the a3r4 and a3r5 treatments. The A4R0 treatment was not significantly different from a4r3 but was significantly different from a4r1, a4r2, a4r4, and a4r5. The a5r0 treatment was not significantly different from a5r1, a5r3, and a5r5 but have shown a significant difference from a5r2, and a5r4. According to Oktafani (2018), plant height is influenced by internal and external factors. Based on the results of analysis of variance there is a link between internal (accession) and external (radiation treatment) factors, where external factors have a very significant effect compared to internal factors. In the induction of chrysanthemum (*Chrysanthemum morifolium)* mutations with a radiation dose of 10-40 Gray, plant height results tend to be lower than the control plants (Yamaguchi *et al*., 2008).

Each plant has own tolerance that is different from growth environment including to sunlight. Some plants grow well in the open area and some grow well with low light intensity or in the shade. Arrowroot is a low light adaptive plant, under the light intensity 7400 lux (27% full light), the number of leaves and tillers were not different under full light, while the plant is higher (Kusmiyati, 2013). According to Suhartini & Hadiatmi (2011), arrowroot plants are plants that would grow optimally under tree stands with minimum light intensity. The results of further tests on the effect of accession on plant height presented in Table 4.

Table 4. Effect of arrowroot accession and radiation on plant height aged 4, 5 and 6 MAP.

Accessions	Average (cm)		
	Month 4	Month 5	Month 6
a1 (Pulosari)	59.46b	78.98c	91.96b
a2 (25 Pandeglang)	58.41b	81.33c	95.92b
a3 (Cikondang)	48.56a	73.50ab	88.45ab
a4 (Tamansari)	54.61b	77.74bc	94.54b
a5 (MN-1)	43.68a	69.73a	87.15a
Radiation doses			
r0 (control)	58.54cd	81.28b	95.08b
r1 (10 Gray)	59.10d	80.56b	94.49b
r2 (20 Gray)	53.70bc	80.59b	94.79b
r3 (30 Gray)	53.08bc	77.70b	96.15b
r4 (40 Gray)	41.10a	58.67a	76.78a
r5 (50 Gray)	52.13b	78.74b	92.32b

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test

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Based on the results in Table 4, it was found that accession of 25 Pandeglang (a2) revealed the maximum height (95.92cm) in observations of 6 MAP, while the shortest was accession of MN-1 (87.15 cm). MN-1 accession was significantly different from Pulosari accession, 25 Pandeglang, Cikondang, and Tamansari.

The effect of radiation doses on plant height showed that the control treatment (r0) was not significantly different from the treatment of radiation doses of 10, 20, 30, and 50 Gray, but show significant differences with the treatment of 40 Gray and 50 Gray. Treatment of 30 Gray radiation dose showed the highest plant height (96.15 cm) at 6 BST observations, while 40 Gray radiation treatment showed the lowest plant height (76.78 cm). Induction of mutations with radiation can cause damage and abnormalities in plant physiology, morphology, and cytology (Kaurr *et al*., 2017).

Table 5. Interaction between accessions and radiation doses on the number of productive tillers and tillers out of sections.

Interactions	Productive Tillers	Tillers out of Section
a1r0	3.00 a	0.94 b
a1r1	2.39 b	0.78 bc
a1r2	1.28 c	0.78 bc
a1r3	2.94 a	0.61 c
a1r4	2.72 ab	1.06 ab
a1r5	2.44 ab	0.89 b
a2r0	1.83 bc	0.78 bc
a2r1	2.33 b	1.06 ab

a2r2	2.94 a	0.61 c
a2r3	2.22 b	0.89 b
a2r4	2.11 bc	1.17 a
a2r5	1.56 bc	0.56 c
a3r0	2.33 b	0.56 c
a3r1	1.72 bc	0.78 bc
a3r2	2.06 bc	0.94 b
a3r3	1.11 c	0.28 c
a3r4	1.06 c	0.00 c
a3r5	1.61 bc	0.28 c
a4r0	1.67 bc	0.39 c
a4r1	1.89 bc	0.83 bc
a4r2	1.50 c	0.39 c
a4r3	2.22 b	1.06 ab
a4r4	1.06 c	0.28 c
a4r5	1.50 c	0.44 c
a5r0	1.50 c	0.28 c
a5r1	1.67 bc	0.22 c
a5r2	0.72 c	0.22 c
a5r3	1.00 c	0.11 c
a5r4	2.06 bc	0.56 bc
a5r5	1.61 bc	0.44 c

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test.

3. The number of productive tillers and tillers out the section

The observations of the number of productive tillers and tillers out of section were carried out when arrowroot plants at 2 - 6 months after planting. The average number of productive tillers and the number of tillers out of section of arrowroot plant in certain accessions presented in Table 5.

From the results showed that the interaction of accessions and radiation doses in the A1R0 treatment gave the most productive number of tillers (3.00) and different from all other treatments (Table 5). While the a5r2 treatment produced number of productive tillers of at least 0.72. The results of the analysis of variance on the number of outgoing tillers were found that the interactions between accessions and radiation doses on the a2r4 treatment showed the most number of outgoing tillers (1.17). Whereas the a3r4 treatment did not obtain the number of tillers coming out of the segment. This is presumably because the arrowroot rhizome used as planting material is an intact rhizome without cutting the tuber into several parts that may lead to producing new shoots.

Table 6. Interaction between accessions and radiation doses on the average length and width of leaves of arrowroot plants.

Interactions	Leaf 's Average Length (cm)	Leaf's Average Width (cm)	Notes
a1r0	14,58bc	5,78bc	
a1r1	19,88c	7,29bc	
a1r2	19,23c	7,71c	
a1r3	20,02c	7,70c	

Interactions	Leaf 's Average Length (cm)	Leaf's Average Width (cm)	Notes
a1r4	18,43bc	7,33bc	
a1r5	21,61c	8,39cd	
a2r0	17,55bc	6,49bc	
a2r1	19,36c	7,84c	
a2r2	15,18bc	6,13bc	
a2r3	16,82bc	6,76bc	
a2r4	16,45bc	6,98bc	
a2r5	12,82ab	5,37b	
a3r0	22,64c	10,17d	
a3r1	16,67bc	6,99bc	
a3r2	19,83c	8,09c	
a3r3	13,79ab	5,51bc	
a3r4	9,08a	3,34a	
a3r5	12,63ab	5,40b	
a4r0	17,33bc	7,21bc	
a4r1	19,39c	7,43c	
a4r2	15,01bc	6,53bc	
a4r3	17,87bc	6,91bc	
a4r4	11,77ab	5,01ab	
a4r5	14,07b	6,01bc	
a5r0	12.96 ab	4.90 ab	
a5r1	15.22 bc	6.20 bc	
a5r2	11.32 ab	4.97 ab	
a5r3	11.32 ab	4.93 ab	
a5r4	15.36 bc	6.27 bc	
a5r5	17.37 bc	6.57 bc	

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test.

Dewanti (2004) and Arifin *et al*. (2014) explained that the difference in cuttings of the base, middle, and the end rhizome that will be used as planting material will affect plant growth and development.

4. Leaf Length and Width

The average length and width of the leaf measured from the 3rd youngest leaf at the top, the leaves' position can be erect or droop or anything in between. Length measurements are carried out from the base of the leaf (on the leaf stem) to the tip of the leaf. The width of the leaf is measured in the middle of the leaf. From the results of observations on the average length and width of the leaves obtained results as presented (Table 6).

From Table 6 can be observed that the interaction between accessions and radiation doses in the a3r0 treatment yield the longest (22.64 cm) and the widest (10.17 cm) leaves. While the a3r4 treatment gave the shortest (9.08 cm) and the smallest (3.34 cm) leaves. The a1r0 treatment (Control) was not significantly different from a1r4 but was significantly different from a1r1, a1r2, a1r3 and a1r5. Glover (2007) stated that the treatment of various doses of gamma irradiation in carnation plants did not cause differences in the vegetative characters of the several genotypes tested, including plant height, leaf number, leaf length, and leaf width.

Based on Table 7, Pulosari (a1) accession has the most length and width of leaves, which are 18.9 cm and 7.37 cm, compared to the accessions of 25 Pandeglang (a2), Cikondang (a3), Tamansari (a4), and MN-1 (a5). On the other hand, the shortest length and width of leaves was found in MN-1 (a5) accession, which are 13.92 cm and 5.64 cm.

Table 7. Effect of accessions and radiation doses on the average leaves length and width.

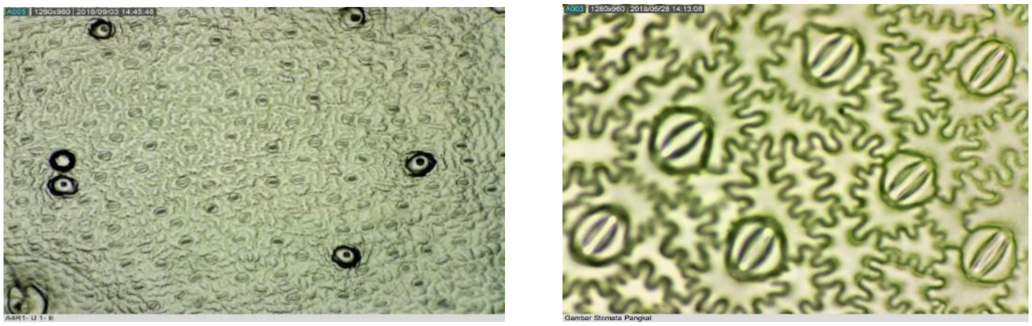
Accessions	Leaves' Average Length (cm)	Leaves' Average Width (cm)
a1 (Pulosari)	18.96 c	7.37 c
a2 (25 Pandeglang)	16.37 b	6.59 b
a3 (Cikondang)	15.78 ab	6.58 b
a4 (Tamansari)	15.91 b	6.52 b
a5 (MN-1)	13.92 a	5.64 a
Radiation Doses		
r0 (control)	17.01b	6.91 b
r1 (10 Gy)	18.10b	7.15 b
r2 (20 Gy)	16.11a	6.69 b
r3 (30 Gy)	15.97a	6.36 ab
r4 (40 Gy)	14.22a	5.79 a
r5 (50 Gy)	15.70a	6.35 ab

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test.

According to Cahyuningdari (2002) and Anggun *et al*. (2018) by increasing number and size of leaves, plants are able to carry out photosynthesis to the maximum to support the transition process towards the reproductive phase. The results of further tests analysis in Table 7 showed that the average leaf length in the control treatment (R0) was not significantly different from the 10 Gray radiation treatment but it was significantly different with radiation treatment of 20 Gray, 30 Gray, 40 Gray, and 50 Gray. The treatment of 10 Gray radiation showed the highest leaf length and width of 18.10 cm and 7.15 cm, while the 40 Gray radiation treatment gave the lowest leaf length and width results of 14.22 cm and 5.79 cm. Maghfoer *et al*. (2003) stated that there is a relationship between leaf area and water availability whereas increasing leaf area would also increase the availability of water. The leaf area is also influenced by genetic factors, the number of leaves and its sizes. Reduced size of plant leaves such as their lengths and widths caused by radiation treatment will reduce the auxin content as far as interfering with auxin synthesis.

Cyto-Morphology Stomata Analysis

The number of stomata was calculated at the end of the observation and was carried out during the day. The leaves (samples) used were the first, the second, and the third leaves which were calculated based on the first leaves that had been fully bloomed on the parent plant. Observations were done using the aid of Nikon light microscope at 10x and 40x magnification, with a field of view of 0.08 mm^2, which could be seen in Figure 4.



A **B**

Figure 4: Arrowroot plant stomata at 10x (A) and 40x (B) magnification

Stomata of arrowroot plants is categorized as a *diasitic* type, where each closing cell is surrounded by two neighboring cells where the dividing wall is perpendicular to the stoma (Kaur *et al*., 2017). The stomata sampled from the bottom of the leaf because the number of stomata on the lower surface of the leaf is greater than the upper surface. As Kaur *et al*. (2017) stated, out of 100 plant species observed, almost all of the monocot and dicot plants that grow on land generally have stomata at the bottom of the leaf surface. Based on the results presented in Table 8 below, it showed that the interaction between accession and radiation for the a5r2 treatment has the least number of stomata which is 81.67. The overall the data have shown that the number of stomata in accession MN-1 (a5) has a fewer number stomata or significantly different from other plant species observed. Almost all of the monocot and dicot plants that grow on land generally have stomata at the bottom of the leaf surface (Sastra 2003., and Mutaqin *et al.*, 2016).

Table 8. Effect of accession interactions and radiation doses on the number of leaves of the arrowroot plant stomata

Interaction Influence	Average
a1r0	140.33b
a1r1	124.00c
a1r2	134.33bc
a1r3	144.67b
a1r4	117.33cd
a1r5	107.67cd
a2r0	139.00b
a2r1	156.33a
a2r2	145.33a
a2r3	131.00c
a2r4	138.33b

a2r5	141.00b
a3r0	130.67c
a3r1	135.67bc
a3r2	119.67cd
a3r3	126.67c
a3r4	133.33bc
a3r5	114.33cd
a4r0	119.67cd
a4r1	126.33c
a4r2	137.67b
a4r3	130.00c
a4r4	138.67b
a4r5	115.33cd
a5r0	89.00d
a5r1	87.67d
a5r2	81.67d
a5r3	82.33d
a5r4	85.00d
a5r5	82.33d

Note: The numbers followed by the same letters are not significantly different at the 5% level according to the Duncan test.

CONCLUSION

1. There were interactions on several quantitative parameters that were being tested i.e. the parameters of growing shoots, plant height, number of tillers, number of tillers out of segments, leaf length, and leaf width.
2. The fastest time for shoot to sprout was 12 days in the accession of Pulosari (a1) with a radiation dose of 0 (a1r0) and 30 Gray (a1r30).
3. The highest plant (106.88 cm) was found in the accession of Cikondang (a3) with a radiation dose of 20 Gray (a3r20) while the shortest is accession to Cikondang (a3) with a radiation dose of 40 Gray (A3R40).
4. The highest number of productive tillers (3.00) was found in the accession of Pulosari (a1) without radiation (a1r0) and the maximum number of out-of-section tillers (1.17) on the a2 (25 Pandeglang) accession and 40 Gray radiation (a2r40).
5. The largest effect of accession interactions and radiation doses on leaf length and width was found in the treatment (a3r0), which was 22.64 cm length and 10.17 cm width. While contrary, the smallest leaf size was obtained from the interaction between Cikondang (a3) accession and the radiation position of 40 Gy (r40).
6. The highest number of stomata was found in the treatment 25 Pandeglang (a2) accession with a radiation dose of 10 Gray (r10), which was 156.33 pieces.

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