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Mutagenesis Effect Using Gamma Ray Radiation on Morphological Changes, Productivity, and Genetic Variation in *Chloris gayana* cv. Callide

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ABSTRACT

Chloris gayana grass is a perennial tropical grass that is highly adaptable and resistant to climate change. As a cover crop, this grass can improve soil conditions. This grass has high palatability and potential to be improved through breeding to enhance the quality and productivity of ruminants' feed. This research was conducted to determine the effect of gamma ray radiation on the morphology, productivity and genetic variation of *C. gayana* cv. Callide. This research used 4 radiation doses of 0, 75, 150 and 225 Gy, which were given to *C. gayana* cv Callide plant seeds via a Gamma ^{60}Co irradiator chamber. A radiation dose of 150 Gy was proven to increase the development of culm diameter ($p < 0.05$). The best productivity was shown by radiation doses of 75 and 150 Gy, which increased the production of fresh, dry, and organic matter. The results of genetic variation analysis using Random Amplified Polymorphic Deoxyribonucleic Acid Polymerase Chain Reaction (RAPD PCR) showed that the polymorphism resulting from using the Organophosphate Degradation (OPD) 8 primer was 66.67%, and the OPD 11 primer was 62.50%. Overall, gamma ray radiation with 75 and 150 Gy doses on *C. gayana* cv. Callide has been proven to improve plant productivity.

Keywords: *Chloris gayana*, gamma ray, genetic variation, plant morphology, plant productivity

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INTRODUCTION

Based on data from the Central Bureau of Statistics Indonesia, the population of beef and dairy cattle increased from 2020 to 2023, but the availability of forage decreased (Badan Pusat Statistik, 2023).

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Challenges in providing forage can come from several factors, including the type and quality of forage varieties, productivity, and ability to adapt to the growing environment. Climate change is also an issue regarding the stability of the current forage availability. Therefore, resilient grass with high adaptability and good nutrient content needs to be enhanced and reproduced; thus, it can meet the needs of ruminant livestock feed.

Chloris gayana grass is included in the cover crop and can grow in tropical and subtropical areas because it is highly adaptable to various environmental conditions. *Chloris gayana* is a perennial grass that can survive for years. That grass has a sturdy structure and strong stolon shape, is resistant to drought, and is able to reproduce quickly using seeds and vegetatively using stems. This plant can be found in grasslands, forests, savannas, riverbanks, lakes and swamps. *Chloris gayana* grass can survive in tropical climates with 6-month dry periods because it has a strong root system that can grow deep down into the soil for 4 meters (Rojas-Sandoval, 2020).

Chloris gayana grass is included in the C_4 plant group, which can carry out photosynthesis efficiently at hot environmental temperatures, making it suitable for use as a protective plant. The formation of grass biomass comes from utilizing soil moisture and absorbing sunlight by 80%. Planting under the shade will cause less optimal growth (Bilgin & Tansi, 2020). Planting *C. gayana* in pasture soil can increase soil organic matter (OM) content and water infiltration capacity, increase water holding capacity and reduce soil temperature during the dry season (Valenzuela & Smith, 2002). The deep root system and its ability to bind soil particles form stable soil aggregates so that the grass can prevent soil erosion (Mulualem et al., 2012).

Chloris gayana grass has good palatability in ruminants (Allah & Bello, 2019). In other studies, it had been reported that using that grass for sheep's feed showed lower palatability compared to *Brachiaria ruziziensis*. Palatability is influenced by the grass's crude fiber (CF) content. High CF will reduce the palatability of feed in ruminant livestock (Kenana et al., 2020). The dry matter (DM) content of *C. gayana* tends to be higher than other types of grass. It affects palatability, which is lower when used as ruminant animal feed. Therefore, the quality and productivity of *C. gayana* grass potentially improved through breeding to meet the needs of ruminant livestock feed.

A plant breeding program employs a structured and systematic approach to improving the genetic characteristics of plants and developing new cultivars with desirable traits. It is a cyclical process, with each cycle comprising three major phases: (1) creating genetic diversity (inducing mutation, making crosses, introducing exotic germplasm and using genetic engineering techniques), (2) assessing and screening to identify superior recombinant (utilizing marker-assisted selection, introducing quantitative trait loci (QTL), using high-throughput phenotyping platforms, resulting in the identification of potential cultivars), and (3) introducing, disseminating and embracing new cultivars. The application of plant breeding has been proven to increase productivity by up to 50% (Ceccarelli, 2015). Plant breeding is carried out by changing the genetic composition of plants so that the desired breeding objectives are achieved (Koryati et al., 2022). This research marks the first step in a plant breeding program targeting the enhancement of genetic diversity in *C. gayana* grass. It used gamma ray radiation for mutagenesis in seeds of *C. gayana* cv. Callide.

Mutagenesis in plant breeding involves artificially increasing mutation frequency to induce genetic changes without genetic segregation or recombination (Raina et al., 2021). Gamma rays, a form of physical mutagen, are effective in altering the genetic structure of mutant plants due to their penetrative nature and ability to break down hydrogen bonds and sugar-phosphate groups within cells (Toker et al., 2007). Gamma-ray radiation causes a plant mutation, altering traits seen through morphological or cell structure changes. This radiation causes the radiolysis of water, producing reactive oxygen species (ROS) by splitting water molecules, leading to oxidative stress (Riviello-Flores et al., 2022).

High doses of gamma ray radiation cause oxidative stress in plants, resulting from an imbalance between ROS production and its enzymatic and non-enzymatic detoxification processes. Elevated ROS production due to high doses of radiation can result in photooxidative damage to Deoxyribonucleic Acid (DNA), proteins and lipids, ultimately resulting in cell death (Tripathy & Oelmüller, 2012). It can cause abnormal development of leaves, flowers, and seeds. Exposure to gamma-ray radiation can induce chromosomal aberrations in mitotic cells, causing chromosomes to become sticky, slow-moving and prone to breakage. Similarly, gamma ray radiation can affect meiotic cells, causing stickiness and disrupted polarity, which may reduce the germination ability of plant seeds (Nurmansyah et al., 2018). At low radiation doses, gamma rays can act as priming radiation, enhancing the germination process and improving cell proliferation, cell growth, stress resistance and productivity yield. The type of plant and the quantity of radiation dose also influence seed germination and seedling growth (Beyaz et al., 2020).

Gamma-ray radiation at doses of 100 to 150 Gy on seeds has been proven to increase germination, root length, the weight of seed yield, DM content and leaf chlorophyll content at the beginning of plant growth. A radiation dose of 50 Gy is known to increase the DM content and fresh weight of seeds compared to other doses (Beyaz et al., 2016). Low-dose radiation 25 Gy can also increase the secondary metabolite content in *Silybum marianum* L plant callus. The interaction between gamma rays and free radicals in plant cells will activate signal molecules in the plant defense system and synthesize plant secondary metabolites (Khalifa et al., 2022).

Gamma-ray radiation can speed up flowering time and produce better agronomic productivity in *Brachiaria*. Applying a dose of 40 Gy yielded the highest dry matter content in *Brachiaria*. Increasing doses of gamma-ray radiation also boosted the number of tillers, leaf-to-stem ratio, leaf length and DM content. However, grass treated with gamma rays showed lower chlorophyll content compared to the control group. Damage to plant pigments due to radiation will cause the loss of the plant's photosynthetic ability. Morphological characteristics are linked to DM yield and plant nutrient quality (Hoka et al., 2019). Furthermore, applying gamma radiation on the nappies grass (*Pennisetum purpureum* Schumach.) increases their morphology and productivity. Furthermore, the impact of the gamma radiation has even produced a new cultivar, namely *P. purpureum* cultivar Gama Umami, whose growth and productivity are higher than its parent Napier grass and the grass produces good quality silage (Ananta et al., 2019; Fahmi et al., 2019; Mudhita et al., 2024; Respati et al., 2018; Umami et al., 2022, 2023).

The appropriate dose of gamma-ray radiation needs to be sought in the breeding process to improve the quality and productivity of *C. gayana* cv. Callide. Gamma-ray radiation at both low and high doses has never been applied in previous studies involving *C. gayana* cv. Callide. The optimal gamma-ray radiation dosage required to enhance the growth and productivity of *C. gayana* cv. Callide will be determined based on the findings of this study.

The effect of mutation on genetic diversity in *C. gayana* cv. Callide must be analyzed to determine the genetic alterations resulting from the gamma-ray radiation process. Random amplified polymorphic DNA (RAPD) analysis, a Polymerase Chain Reaction (PCR)- based method, can identify variations or polymorphism induced in plants by radiation. RAPD PCR analysis can reveal genetic changes in *C. gayana* cv. Callide is caused by gamma ray radiation, allowing the assessment of the relation between irradiated and non-irradiated grass. Anggereini (2008) stated that the RAPD analysis can quickly and effectively identify genetic markers to distinguish between closely related and morphologically indistinguishable species. The RAPD marker is used to create genetic maps identifying strains, species, populations and systems of various organisms. Therefore, an RAPD-PCR analysis was also conducted to identify the genetic variation that occurred. In this study, the effect of mutagenesis using gamma-ray radiation is determined through the morphological parameters, productivity and genetic variations observed.

MATERIALS AND METHODS

Material Preparation

This research was conducted from January to May 2023 in the greenhouse area of Forage and Pasture Laboratory, Faculty of Animal Science, Universitas Gadjah Mada, Sleman, Special Region of Yogyakarta Province, Indonesia. *Chloris gayana* cv. Callide seeds were purchased from Crop Mark Seed Company, New Zealand, without detailed harvest time and seed content information. Gamma ray radiation on *C. gayana* cv. Callide seeds was carried out using IRPASENA 4000 A gamma isotope Cobalt-60 chamber at the Research and Development Center Laboratory for Isotope and Radiation Technology, National Nuclear Energy Agency of Indonesia (BATAN). Seeds were given radiation with doses of

0 (P0), 75 (P1), 150 (P2) and 225 Gy (P3). Irradiated seeds are selected seeds that have good quality. Seeds are put in plastic and labeled with each dose of radiation number, then irradiated in a Gamma Chamber irradiator (IRPASENA BATAN, Indonesia) with a dose rate of 10Gy/87 seconds. The dose size is a function of time, and the dose rate of the Gamma Chamber is at that time.

Planting was carried out for 90 days at the greenhouse area of the Forage and Pasture Laboratory, Faculty of Animal Science, Universitas Gadjah Mada. Planting preparations included germination and preparation of planting media. Germination is carried out to ensure that the seeds planted are good quality seeds that grow successfully. The planting medium used in this research was a mixture of soil, manure, and bamboo humus in a ratio of 2:1:1 in polybag media. Maintenance, weeding and fertilization were carried out during the planting period. Plant maintenance included watering every morning and evening, as well as weeding to remove weeds. Fertilization is carried out on the 30th day after planting (1 kg/ha).

Experimental Design

This study used a completely randomized design with one primary factor of gamma irradiation, three levels of treatment and one control group. Plants were moved to polybag media two weeks after germination. One plant was planted in each polybag. Plants were grouped based on radiation doses of 0 (P0) or control, 75 (P1), 150 (P2) and 225 Gy (P3). The four groups of plants are evenly placed to receive the same environment in the greenhouse. Each group of plants had 20 replications, so the total number of polybags in this study was 80. Samples from each plant in each polybag were used to collect data on morphological and productivity parameters.

Data Collection of Morphological Parameters and Productivity

Morphological measurements were carried out at harvest, 90 days after planting. Morphological variables measured include plant height and length, number of leaves, leaf length and width, culm diameter growing and creeping on the ground and number of tillers. Plant height was measured starting from the ground surface to the tallest leaf. Plant length was measured starting from the soil surface to the longest leaf. The number of leaves was observed by counting the green leaves on each plant. The length of the leaves was observed by measuring the base to the tip of the leaf on each plant. Leaf width is the longest extension of any two points on the blade edge of the leaves. Creeping culm diameter is the size of the diameter of the culm that grows as a vine. Growing culm is culm that grows upwards. The number of tillers was calculated by counting each polybag's shoots.

Grass productivity is measured in fresh, DM, and OM production. Fresh production was determined by weighing all parts of the defoliated plant. Plant weight at harvest (g/polybag) was converted into units of t/ha, then multiplied by the percentage of DM to determine the DM production. The results of DM production calculations (t/ha) are multiplied by the percentage of OM to get the total value of organic matter production. The DM and OM contents were determined using the method explained by Association of Official Agricultural Chemists (2005).

RAPD PCR ANALYSIS

RAPD PCR analysis was carried out using leaves by extracting, quantifying, and diluting the DNA. Leaves were washed with running water until clean and dried with tissue. DNA extraction using 0.1 gram of leaves concentration was then used for the PCR process. This analysis uses ten samples of *C. gayana* cv. Callide (Table 1). Quantification of DNA uses Gene Quant to determine the DNA concentration and DNA-RNA ratio, which was obtained by measuring light absorption at a wavelength of 260 nm. Next, DNA dilution was carried out by adding ddH$_2$O solution to obtain a suitable concentration for amplification.

Amplification of DNA was carried out using primers OPD 8 and OPD 11 (Table 2) with the nucleotide base sequences listed in Table 3. PCR reactions were carried out in a total volume of 10 µl for each PCR tube. Each PCR reaction consisted of 5 µl PCR mix Go Taq® Green (Promega, USA), 0.25 µl 100 µM primer (Sigma-Proligo, Germany), 2.5 µl DNA sample and 2.25 nuclease-free water. DNA amplification was carried out using the BOECO PCR System. First, heating was carried out at 94°C for 30 seconds, annealing at 37°C for 30 seconds, and elongation at 72°C for 1 minute 30 seconds, followed by final elongation at 72°C for 7 minutes. The DNA resulting from the PCR was then electrophoresed using 1.0% (w/v) agarose, which had been added with florosafe DNA stain as a dye, in TBE buffer (which consisted of 0.45 M Tris-HCl pH 8, 0.45 M Boric acid, 20 mM EDTA) with a voltage of 100 volts for 45 minutes. The amplification results are then visualized with UV light.

Table 1
List of Chloris gayana *cv. Callide samples for RAPD PCR analysis*

No	Sample name	Sample code
1.	P0A1	A1
2.	P1A1	A2
3.	P1A2	A3
4.	P1A3	A4
5.	P2A1	A5
6.	P2A2	A6
7.	P2A3	A7
8.	P3A1	A8
9.	P3A2	A9
10.	P3A3	A10

Table 2
Levels of polymorphism in each of the OPD 8 and OPD 11 primers

Primer	Amplified loci	Polimorphic loci	Polimorphic loci (%)
OPD 8	9	6	66.67
OPD 11	8	5	62.50

Table 3
List of primers used in DNA amplification

Primer	Sequence of nucleotide bases
OPD 8	GTGTGCCCCA
OPD 11	AGCGCCATTG

Statistical Analysis

Production yield and morphological parameters of *C. gayana* cv. Callide were analyzed using completely randomized designed One-Way Analysis of Variance (ANOVA). Mean comparisons were conducted using the Duncan Multiple Range Test at $p < 0.05$. Genetic diversity data was obtained by scoring the electrophoresis results for each individual at a certain size; if a band appeared, they were given a score of = 1, and if no band appeared, they were given a score of = 0. Binary data was then analyzed with Genalex 6.1 to determine the occurring polymorphism.

RESULTS AND DISCUSSION

Plant Morphology

The statistical analysis results in this study show plant height, number of leaves, culm diameter, and number of *C. gayana* cv. Callide tillers exhibited significant differences ($p < 0.05$) among treatments. The results for plant height, number of leaves and number of tillers indicate that grass without gamma-ray radiation yielded significantly higher results ($p < 0.05$) compared to grass treated with 75, 150 and 225 Gy radiation doses. The highest culm diameter was observed at a radiation dose of 150 Gy ($p < 0.05$). Plant length, leaf length and leaf width did not show any statistical differences ($p > 0.05$) among treatments. Data on growth measurement results are presented in Table 4.

Plant height and length are formed through cell division and elongation during the physiological phase. The activity of auxin influences the increase in plant height and length. Results of this study showed that the height of *C. gayana* cv. Callide without gamma-ray radiation was significantly ($p < 0.05$) higher than grass treated with radiation, reaching 72.13 cm. In contrast, the length of the grass *C. gayana* cv. Callide did not show a significant

Table 4
Morphological characteristic data of Chloris gayana *cv. Callide with different radiation doses*

Morphological characteristics	Gamma ray radiation dose (Gy)			
	0	75	150	225
Plant height (cm)	72.13 ± 2.88^{a}	52.36 ± 4.23^{b}	42.81 ± 3.62^{b}	52.38 ± 5.10^{b}
Plant length (cm)	170.35 ± 3.40	159.50 ± 6.64	168.51 ± 5.73	167.03 ± 8.21
Leaf number	183.05 ± 3.05^{a}	92.25 ± 5.99^{bc}	114.73 ± 14.63^{c}	75.44 ± 7.02^{c}
Leaf width (mm)	6.82 ± 0.33	7.77 ± 0.25	7.74 ± 0.30	7.07 ± 0.32
Leaf length (cm)	68.63 ± 3.13	87.38 ± 33.38	52.63 ± 1.46	56.56 ± 2.65
Creeping culm diameter (mm)	3.10 ± 0.05^{c}	4.16 ± 0.06^{b}	5.04 ± 0.02^{a}	4.24 ± 0.04^{b}
Growing culm diameter (mm)	2.18 ± 0.08^{c}	3.53 ± 0.06^{b}	4.04 ± 0.04^{a}	3.41 ± 0.04^{b}
Tiller number	102.00 ± 1.06^{a}	53.60 ± 6.41^{c}	76.78 ± 8.71^{b}	46.77 ± 6.45^{c}

Note. ^{a,b,c} Different superscripts in the same row indicate a different significance ($p < 0.05$)

difference ($p > 0.05$) in this study. According to Valenzuela & Smith (2002), the height of *C. gayana* grass can generally reach 50 to 200 cm. The research results of Jabessa et al. (2023) reported that planting *C. gayana* grass in the high and midlands in the Guji region, Ethiopia produced different plant heights. The height of *C. gayana* in the highlands was 106.8 cm, while in the lowlands, it reached 172.1 cm. Research conducted by Mohamed & Gebeyew (2018) on planting *C. gayana* showed that the plants matured on the 87th day, marked by flowering reaching 50% and a height of 139.10 cm. This site is reported to be significantly higher than buffalo grass and *Panicum maximum*.

The height of *C. gayana* grass planted on savanna land in Ethiopia at 8 weeks reached 100.7 to 121 cm. Based on research conducted by Daba et al. (2019), *C. gayana* harvested 75 days after planting showed plant height reaching 93 to 120 cm in each ILRI-7384 and ILRI-6633 varieties. Mganga et al. (2015) reported that rapid plant growth and development can result from a faster germination process. High doses of radiation will cause changes in the ratio of the auxin and cytokinin, leading to alteration in cell differentiation patterns. An increase in plant height can occur when radiation is given at doses of 10 and 30 Gy, while higher doses will reduce plant height. Increasing the radiation dose will damage the chromosome structure, thereby reducing plant height. Hartati et al. (2021) stated that physiological damage due to gamma-ray radiation may include cell death, inhibition of the cell division process and changes in plant reproductive characteristics. Gamma-ray radiation can increase plant height if administrated at the right dose because it induces hormonal changes as a result of increasing stress factors in plants (Singh et al., 2019). Taller plants are capable of producing greater biomass due to the stronger structure of their stems and shoots (Joshi et al., 2016).

All the leaves on each *C. gayana* cv. Callide were counted, and the results of this study were reported. The number of leaves in this study showed significant differences ($p < 0.05$) among treatments. *Chloris gayana* cv. Callide without seed radiation produced the highest number of leaves, 183.05. Meanwhile, the number of leaves resulting from the radiation of 75, 150 and 225 Gy were 92.25, 114.73 and 75.44, respectively (Table 4). These results are consistent with research on gamma-ray radiation given to chili plants. Treatment without radiation resulted in the highest number of leaves compared to 100, 200 and 300 Gy radiation (Tias et al., 2022). Increasing the radiation dose will prolong the exposure time to gamma rays, thereby increasing the cellular damage from the radiation energy (Makhziah et al., 2017).

The correct radiation dose can increase the number of leaves on plants due to increased tissue differentiation from the gamma ray radiation process. Gamma-ray radiation using Cesium-137 on soybean seeds can increase the number of leaves at a dose of 75 Gy, while lower and higher doses have been shown to reduce the number of leaves (Nuraeni et al., 2023). Gamma ray radiation using Cobalt-60 at a dose of 10 Gy was also proven to increase the number of leaves compared to treatment without radiation in *Amorphophallus muelleri* plants (Santosa et al., 2014).

The activity of the auxin influences the growth of leaf width and length. This study's leaf width and length measurements showed no differences ($p > 0.05$) among treatments. The sizes of the leaf width resulting from gamma ray radiation with doses of 0, 75, 150 and 225 Gy, respectively, were 6.85, 7.77, 7.74 and 7.07 mm (Table 4). Meanwhile, the length of each leaf was 68.63, 87.38, 52.63 and 56.56 cm, respectively (Table 4). Leaf size is important for plants because it affects the amount of chlorophyll available for the plant's photosynthesis process. Non-optimal photosynthesis reactions can be influenced by a small amount of chlorophyll, which can cause plant growth to be hampered (Sarah et al., 2023). Gamma ray radiation with a dose of 20 Gy can produce the longest leaves compared to treatment without radiation and another dose on the ambon banana plant (*Musa paradisiaca* var. sapientum). Gamma ray radiation did not have a significant effect on leaf width (Due et al., 2019). Radiation doses that are too high are likely to damage the mobilization of nutrients from seeds to leaves (Santosa & Sugiyama, 2007). Longer leaf life is a desirable characteristic to maximize plant nutrient production. Cytokinin hormones influence leaf fall time, so damage to these hormones will result in less optimal plant production (Santosa et al., 2014).

The creeping culm of *C. gayana* is part of the vegetative reproductive organs; meanwhile, erect culm results from plant growth and is influenced by auxin activity. The Culm of the plant also determines the amount of biomass and crude fiber contained in the plant. Results of culm measurements on *C. gayana* cv. Callide from this research showed that gamma ray radiation has a significant effect on the diameter of creeping culm and growing culm ($p < 0.05$). A radiation dose of 150 Gy gave the biggest diameter, 4.04 mm in growing and 5.04 mm in creeping culms. Radiation of 75 and 225 Gy produced higher diameter sizes compared to treatment without radiation. The diameter of the growing stem from radiation results of 0, 75 and 225 Gy, respectively, were 2.18, 3.53 and 3.41 mm, while the respective creeping diameters were 3.10, 4.16 and 4.24 mm, respectively (Table 4). Each part of the organ in a plant provides a different biological function. The leaf part of the plant has the function of forming carbohydrates; the root function is to absorb water and nutrients, while the culm has the mechanical function of transporting nutrients and storing nutrient reserves for the plant (Poorter et al., 2012).

The development of tiller is influenced by the activity of auxin and cytokinin in plants. It is important to know tiller development because it affects the productivity and reproductive ability of the grass through the formation of flowers. The statistical analysis results in this study showed that gamma-ray radiation on *C. gayana* cv. Callide significantly reduced the number of tillers that developed ($p < 0.05$). Several tillers of *C. Gayana* cv. Callide without radiation were 102 segments, while radiation of 75, 150, and 225 Gy each produced 53.60, 76.78, and 46.77 tiller segments, respectively (Table 4).

Tillers determine the amount of plant biomass. The number of tillers per plant in *C. gayana* for 75 days of planting reached 5.4 to 9.4 segments, depending on the varieties (Daba et al., 2019). The bigger number of tillers will produce higher non-structural carbohydrate components because they increase the number of leaves. It is in accordance with the aim of grass breeding to increase the biomass content and quality of the grass. *Chloris gayana* is able to produce a high number of tillers so that it has better productivity and vegetative growth (Tadesse et al., 2022).

Warid et al. (2017) reported that higher radiation doses reduced the survival ability of soybean plants, especially at a dose of 400 Gy. Wiryosimin (1995) also reported that gamma ray Co-60 radiation could produce high energy, damaging the chemical bonds of a new compound when given to seeds, plant tillers, pollen, apical shoots, plant tissues and cells. Radiation of gamma ray Co-60 in seeds can cause the cell nucleus to experience genome mutations, chromosome mutations, gene mutations or mutations outside the nucleus, such as in the plastids and mitochondria. Genomic mutations will cause changes in the number of chromosomes. The addition or reduction of chromosome cells will result in changes in the characteristics and morphology of plants in the form of height, number of leaves and number of tillers. Harmini et al. (2021) stated that 50 Gy gamma ray radiation on *Pennisetum purpureum* cv Taiwan produced a lower number of tillers in 90% of the replications compared to the control. Higher radiation doses can reduce these plants' growth tiller, leaves and roots.

Gamma ray radiation provides random results on the influence of the metabolism of cells exposed to the radiation process. Gamma rays can influence meristem cell metabolism and protein synthesis during stem development. The radiation process causes the formation of free radicals, which react with organic molecules, thereby disrupting cell metabolic processes (Hartati et al., 2021).

Plant Productivity

This research shows that gamma ray radiation influenced fresh yield, DM yield and OM yield of *C. Gayana* cv. Callide ($p < 0.05$). The highest fresh yield and OM yield were obtained at a radiation dose of 150 Gy, while the highest DM yield was obtained at a radiation dose of 75 and 150 Gy. Production results of *C. gayana* cv. Callide treated with gamma ray radiation is provided in Table 5.

Production of fresh biomass from *C. gayana* cv. Callide in this study was proven to be significantly influenced by gamma ray radiation ($p < 0.05$). Radiation doses of 75 and 150 Gy gave the highest results (2.38 and 2.61 t/ha, respectively). Treatment without radiation gave a fresh yield of 1.97 t/ha, and the lowest production was produced by a radiation dose of 225 Gy (1.35 t/ha) (Table 5). Biomass allocation to plant morphological components gives varying results. Biomass calculations are often carried out on feed plants' leaf and

Table 5

Production of fresh yield, dry matter yield and organic matter yield of Chloris gayana *cv.* Callide *with different radiation doses*

Yield (t/ha)	**Gamma ray radiation dose (Gy)**			
	0	**75**	**150**	**225**
Fresh yield	1.97 ± 0,09^{b}	2.38 ± 0,10^{a}	2.61 ± 0,09^{a}	1.36 ± 0,10^{c}
Dry matter yield	0.31 ± 0,02^{b}	0.38 ± 0,02^{ab}	0.48 ± 0,02^{a}	0.18 ± 0,01^{c}
Organic matter yield	1.61 ± 0,08^{b}	1.99 ± 0,08^{a}	2.21 ± 0,08^{a}	1.15 ± 0,09^{c}

Note. ^{a,b,c} Different superscripts in the same row indicate a different significance ($p < 0.05$)

stem ratio (Poorter et al., 2012). Based on studies using allometric equations on biomass in plants' roots, stems and leaves, it was reported that increasing plant size causes biomass allocation to the stem to increase and the leaves to decrease (Liu et al., 2021). It is in accordance with the results of this study that the highest production at radiation doses of 75 and 150 Gy was due to the highest culm diameter obtained at these radiation doses (Table 4).

Kebede and Bobo (2023) reported that fresh biomass production in *C. gayana* was influenced by the type of variety and altitude of the planting area. *Chloris gayana* planted in the highlands produces lower fresh biomass production than when planted in the lowlands. Production of fresh biomass from *C. gayana* cv. Masaba and cv. ILRI-7384 in the highlands were 3.45 and 3.25 t/ha, while when planted in the lowlands, it produced 4.24 and 4.08 t/ha, respectively. Hidosa et al. (2018) reported that the fresh biomass production of Chloris Gayana grass on irrigated land was 53.56 t/ha/year. Giving gamma ray radiation will increase cell proliferation, but at high doses, it can damage cells and inhibit plant growth.

Gamma ray radiation in this study was proven to significantly influence the DM yield of *C. gayana* cv Callide ($p < 0.05$) among treatments. The radiation dose treatment of 150 and 75 Gy gave the highest results (0.48 and 0.38 t/ha, respectively), while the radiation dose treatment of 225 Gy produced the lowest DM yield (0.18 t/ha). Treatment without radiation gave results of 0.31 t/ha (Table 5). These results are lower than those Abera (2017) reported in that the DM production of *C. gayana* planted together with *Medicago sativa* could reach 3.90 to 4.44 t/ha. *Chloris gayana* is a perennial plant that can grow for up to 3 years with 2-week harvest interval. Jabessa et al. (2023) reported that planting *C. gayana* in the highlands resulted in a DM production content of 8.94 t/ha/year while planting in the midlands resulted in a DM production content of 13.34 t/ha/year. Increasing the dose of gamma ray radiation can cause dehydration or changes in the ratio of meristem cells and special plant cells, which include root hair cells, palisade, xylem, and phloem. It causes division in small cells and is detrimental to large vacuolated cells, which accumulate secondary metabolites so that DM production will increase. Each plant species has a different response to gamma ray radiation. Several studies related to gamma ray radiation show that giving a radiation dose of 40 Gy will cause a decrease in growth indicators in plants (Ciocan et al., 2023). Furthermore, Respati et al. (2018) reported that *Brachiaria brizantha* cv. MG5, germinated from the seeds, radiated using gamma radiation at 100 Gy, resulting in the best growth and production at the regrowth phase 2.

Chloris gayana cv. Callide radiated by gamma ray in this study was proven to significantly influence the results of Organic Matter (OM) yield production ($p < 0.05$). Radiation doses of 75 and 150 Gy resulted in the highest OM production (1.99 and 2.21 t/ha, respectively). Meanwhile, a radiation dose of 225 Gy resulted in the lowest OM production (1.15 Gy) (Table 5). Delastra et al. (2021) stated that a gamma ray radiation dose of 300 Gy to *Sorghum sundanese* plants was proven to increase the DM and OM content.

Changes in plant OM production due to gamma ray radiation are caused by disturbances in cellular metabolism caused by oxidative stress, which reacts with all structural and functional organic molecules, including proteins, lipids and nucleic acids. This reaction causes lipid membrane peroxidation, thereby disrupting membrane stability and increasing permeability. It may cause cell damage and disruption of plant physiological functions, such as changes in plant physiology, increased respiration, increased ethylene production and changes in enzyme activity (Marcu et al., 2013).

Genetic Variation Using RAPD PCR

Amplification results from the RAPD PCR analysis on *C. gayana* cv. Callide, using primers OPD 8 and OPD 11 (Table 3), showed that all plant samples produced clear bands, which can be seen in Figures 1 and 2. The samples that amplified the most and thickest bands originated from plants irradiated with a dose of 150 Gy, specifically samples A5, A6, and A7. The appearance of the amplification band, which differs from that of the control group, indicates a genetic change resulting from the gamma ray radiation in this study.

Genetic diversity testing with RAPD PCR involves primer selection. Primers suitable for RAPD PCR analysis must meet certain criteria: they should generate polymorphic DNA band, yield clear and reproducible results, exhibit stable DNA band amplifiationc, be easy to interpret, and possess a G=C base pair content between 60-70% (Hartati et al., 2007). The success of the PCR test is also influenced by factors such as DNA concentration and template, annealing temperature, and concentration and quality of the primers and buffers used. A DNA template concentration that is too low will result in unclear or absent amplification of DNA bands (Setyawati & Zubaidah, 2021). The freshness level of the leaf samples could influence the differences in the thickness of the bands observed. Fresh samples produce thicker bands (Siregar & Diputra, 2013). Based on the result of the primer selection conducted in this study, only the OPD 8 and OPD 11 primers displayed DNA bands clearly. Therefore, these primers were chosen for RAPD PCR analysis.

Based on the analysis conducted in this research, it is evident that the OPD 8 primer produced 66.67% polymorphic loci, while the OPD 11 primer produced 62.50%

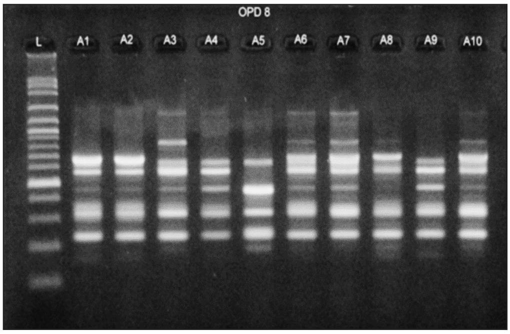

Figure 1. RAPD profiles amplified from 10 Chloris gayana cv. Callide plant using primer OPD-8
Note. A1=Control group plant; A2-A3=Seeds irradiated with 75 Gy; A4-A7=Seeds irradiated with 150 Gy; A8-A10=Seeds irradiated with 225 Gy

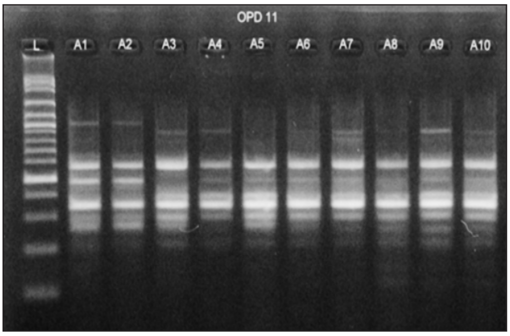

Figure 2. RAPD profiles amplified from 10 Chloris gayana cv. Callide plant using primer OPD-8
Note. A1=Control group plant; A2-A3=Seeds irradiated with 75 Gy; A4-A7=Seeds irradiated with 150 Gy; A8-A10=Seeds irradiated with 225 Gy

polymorphic loci, resulting in a total of 11 polymorphic loci. Sulistyawati and Widyatmoko (2017) stated that the higher number of polymorphic loci resulting from RAPD PCR analysis represents high heterozygosity and heterogeneity. This research indicates that gamma ray radiation induces mutant plants with a moderate level of diversity compared to the control group without radiation treatment. Diversity values are divided into three categories: low (0.1 to 0.4), moderate (0.5 to 0.7), and high (0.8 to 1.0) (Nei, 1987). Greater genetic diversity within a population leads to increased availability of germplasm for developing new varieties (Gusmiaty et al., 2016).

Gamma ray radiation applied to ginger plants at doses of 0, 5, 7, 9, 11 and 13 Gy resulted in a 98.29% polymorphism percentage. The high levels of polymorphism indicate a significant alteration in the DNA sequence of mutant plants resulting from radiation, as influenced by the primer binding used (Mohd Sharim & Shamsiah, 2021). The RAPD PCR analysis was conducted on *Oryza sativa* L. cv. Barak Cenana plants, using five primers, and subjected to gamma ray radiation doses of 100, 200, 400 and 800 Gy, produced six polymorphic bands. Changes in DNA structure, such as transposition, breaks or deletions, can lead to the emergence of new DNA bands as a consequence of induced mutations. The plants resulting from 100 Gy radiation showed the highest level of similarity to the plants without radiation, with a similarity index of 0.75. The lowest level of similarity was observed in the plants resulting from 400 Gy radiation, with a similarity index of 0.33 (Pujiyanti et al., 2021). Genetic diversity testing using RAPD analysis on four samples of ramie plants resulted in a polymorphism percentage ranging from 81% to 100% (Maryeni et al., 2019). The results reported in the literature are significantly higher than those obtained in this study.

Based on RAPD PCR analysis, gamma ray radiation was reported to increase genetic diversity and genetic distance values in *A. muelleri* plants. Gamma ray radiation produced random results; therefore, the appearance of DNA bands in RAPD analysis is not concentrated at low or high radiation doses. Damage to the DNA double helix occurs due to chemical changes caused by radiation, leading to breaks in the DNA molecular chain. The amplification of DNA fragments in RAPD PCR analysis depends on the primer sequence in the DNA genome. The RAPD technique can detect changes in a single base in genomic DNA. Hence, a difference in one nucleotide will produce a distinct RAPD profile. (Poerba et al., 2009). The results of the RAPD PCR analysis in this study indicated a general increase in polymorphism in plants resulting from radiation, although it remained within the moderate category.

CONCLUSION

Based on this research, a 150 Gy gamma ray radiation dose in *C. gayana* cv. Callide showed the best results in the culm diameter, while 75 and 150 Gy gamma ray radiation doses produced the best yields of fresh, DM and OM. Analysis of RAPD PCR shows that plants resulting from 150 Gy radiation produce the best results that can be used for the further breeding process in *C. gayana*.

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