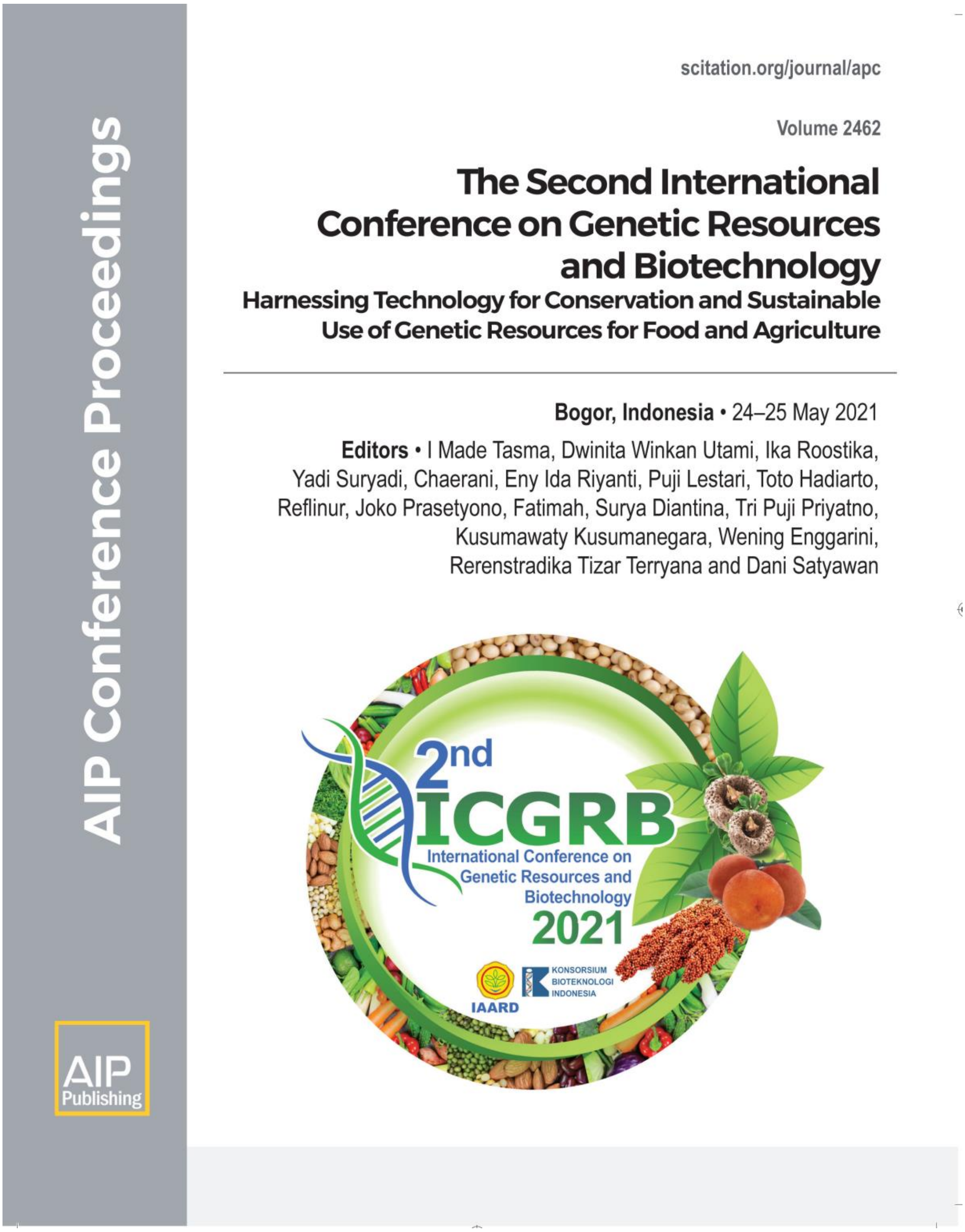

January 2022

THE SECOND INTERNATIONAL CONFERENCE ON GENETIC RESOURCES AND BIOTECHNOLOGY: Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture

Committees: The Second International Conference on Genetic Resources and Biotechnology

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Preface: The Second International Conference on Genetic Resources and Biotechnology

The Second International Conference on Genetic Resources and Biotechnology, which is the continuation of the first event held in 2018, focuses on topics related to advances in biotechnology to create more opportunities for effective conservation and sustainable utilization of genetic resources for food and agriculture. This year conference's theme is Harnessing Technology for Conservation and Sustainable Use of Genetic Resources for Food and Agriculture. The conference was organized by Indonesian Agency for Agricultural Research and Development (IAARD), Ministry of Agriculture, Indonesia, in collaboration with Indonesian Biotechnology Consortium and held on 24^{th}-25^{th} of May 2021virtually due to the pandemic of COVID-19.

The conference aims to share and exchange current scientific information and technological developments on biotechnology and their applications for conservation and sustainable use of genetic, to encourage and promote quality, efficiency, and modernization of management and utilization of genetic resources, and to facilitate national and international collaboration among participants. There are five scopes discussed in this conference. They are effective management of conservation and sustainable use of genetic resources for food and agriculture, application of genomics and molecular markers for genetic resource conservation and crop adaptation to climate change, application of innovative crop improvement techniques for conservation and sustainable use of plant genetic resources for food and agriculture, plant cell and tissue culture for conservation and effective utilization of genetic resources, and the use of microbial genetic resources as biological control agents of agricultural pests and diseases, and for soil bioremediation.

Five speakers from the United States of America, Japan, India and Indonesia were invited to discuss about their expertise and knowledge on relevant subjects in the plenary sessions. This conference was attended by more than 100 participants including 75 presenters and 44 listeners worldwide. They came from diverse governmental, private, or academic institutions and also scientific communities. The presented materials have undergone peer review processes and only qualified papers were selected. Furthermore, all papers were subjected to double blind peer-review and expected to meet the scientific criteria of significance and academic excellence to be published in a conference proceedings indexed in a well-known, reputable service.

We would like to express our sincere gratitude to our speakers, presenters and all participants for their contributions in this conference. We would also like to express our appreciation for the generosity of our sponsors that support this conference: PT CropLife, PT ITS Science Indonesia, PT Fajar Mas Murni and PT Prima Instrument Analitika. Lastly, special thanks to all committee members for their exceptional work and contributions in the conference and publication.

Chair of Organizing Committee

Dr. Toto Hadiarto

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Field evaluation of elephant grass mutant lines (*Pennisetum purpureum* Schumach.) in highlands

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Ali Husni, Muhammad Rifay, Mia Kosmiatin, et al.

Field Evaluation of Elephant Grass Mutant Lines (*Pennisetum purpureum* Schumach.) in Highlands

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Abstract. Forages plays a pivotal key to run livestock business successfully. Elephant grass cv. Taiwan is one of high quality forages with high productivity and nutrient content. Planting the elephant grass in highlands has not been widely applied, hence, the mutation induction techniques can produce mutants with new superior characteristics to adapt in the highlands. This study aimed to evaluate yield characters and nutrient contents of the elephant grass mutant lines in Cijeruk, Bogor. The research used randomized block design (RBD) with nine treatments and three replications. The results showed that each elephant grass mutant line had different response to each parameter. The best growth rate, productivity, and quality was obtained from M7 line with the highest parameters on plant height (226.86 cm), stem length (142.91 cm), stem diameter (2.13 cm), crude protein content (PK; 17.50%), and crude fiber (32.01%).

INTRODUCTION

Elephant grass (*Pennisetum purpureum* Schumach.) is tropical forage originally from Africa with high production capacity and accumulation of high quality dry matter [1]. In this modern era, this grass has attracted many attentions from developed countries related to the development of bioenergy sources because of high biomass yields (67−93 t/ha), low production costs, and no competition with food for human [2]. Aside from being a source of bioenergy, elephant grass is also potential as a forage for ruminants. In Brazil, elephant grass is widely used as a source of feed for dairy cattle [3]. In South America, the grass has been used as animal feed for long time, thus, various attempts have been done to determine the palatability and nutritional value as animal feed [4]. Currently, elephant grass is considered an invasive species in the United States due to the absence of natural enemies to control the growth and population dynamics [5]. In line with the program by Indonesian Government targeting the self-sufficiency on beef in 2026 and becoming the world's food barnstead in the Grand Design of World Food Barn 2045, the Government has been running a Special Efforts Program called SIWAB "*Sapi Indukan Wajib Bunting*" or "Mother Cow must be Pregnant". This effort showed its commitment to increase beef cattle population and meat sufficiency in 2026 [6]. The current main obstacle faced by farmers deals with the fulfilment of continuous supply of high quality forage as good feed. Forages plays a pivotal key to run livestock business successfully, taking about 70% of production costs in maintaining livestock commodities [7]. The improvement of productivity and quality of forages can be done by increasing the genetic potential of elephant grass [8].

Elephant grass cv. Taiwan is a type of forage widely grown until this time because it has high productivity reaching about 300 t/ha/year. This grass has a large size and fluffy leaves, soft stems, 4−5 m height, and good nutrient content. In addition, this grass has a special characteristic, namely the young stem at the base of the bottom is reddish in color. However, this grass cannot survive in areas of continuous rain and dry areas [9]. Development of elephant grass for highlands has not been done considerably. Therefore, inline with the developed science in modern

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genetics, plant breeding has been into a solution of how a plant variety could obtain superior character and properties from genetic engineering. The technique on mutation induction could support the acquisition of new mutant varieties that are beneficial for the business purposes. Genetic materials in this research come from the collection of Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD/BB Biogen), including mutant strains of elephant grass cv. Taiwan as a result of the mutation induction during the callus phase irradiated with gamma rays. This study aimed to evaluate yield characters and nutrient contents of the elephant grass mutant lines in Cijeruk, Bogor, West Java.

MATERIALS AND METHODS

The experiment was carried out in the village of Tajur Halang, Cijeruk District, Bogor Regency, West Java, Indonesia at an altitude of 772 m a.s.l., from June to August 2020. The materials consisted of cuttings of eight elephant grass mutant lines (M1, M2, M3, M4, M5, M6, M7, and M8) derived from mutation induction in the callus phase irradiated with gamma rays, and the wild type elephant grass cv. Taiwan. Land preparation includes cultivating land, setting plots of land, and planning the layout of the experiment. Soil analysis was carried out by taking a sample of 1 kg of soil from a depth of 0–20 cm at five points, then mixed and analyzed in the Soil Testing Laboratory of the Indonesian Soil Research Institute, Bogor. A week before planting, each hole was given manure at a dose of 20 t/ha. Each cutting consisted of two segments with the base cut at an angle and the top is flat. The cuttings were planted on the experimental plots at an angle of 30 degrees at a planting distance of 75 cm × 100 cm, 100 cm distance between rows, 150 cm distance between replications. Soil analysis was carried out by taking a sample of 1 kg of soil from a depth of 0−20 cm at five points then mixed and analyzed in the Soil Testing Laboratory. The research used a single factor randomized block design (RBD) with three replications. Each treatment consisted of 30 groves of plants, so the total experimental units was 810 plants. The second fertilization was carried out 3 weeks after planting using NPK fertilizer at a dose of 200 kg/ha. Plant maintenance included periodic weeding and piling which was carried out simultaneously with fertilization. Defoliation was carried out 60 days after planting (DAP) by cutting the stem at 5−10 cm above the soil surface. From each selected line, 400 g samples were taken, cut into pieces, dried in an oven at 60°C for 48 h, and then ground into flour, prior to proximate analysis. The proximate analysis was done to determine the nutrient content, i.e. crude protein (CP), crude fiber (CF), fat (F), water content (WC), and ash content (AC), in the Research Center for Bioresources and Biotechnology, IPB University. Agronomic data was analyzed statistically using ANOVA and if the results showed significantly different effects, then further test using Duncan's Multiple Range Test (DMRT) at the 5% level was applied.

RESULTS AND DISCUSSIONS

The result showed significant differences in each parameter as a whole, each elephant grass mutant line showed a different response to the observed parameters (Table 1). The difference in response was due to the effect of mutation, environment, and the characteristic of the lines.

TABLE 1. Results of analysis of variance of observed parameters of elephant grass mutant lines at 60 days after planting.

No.	Parameters	F-value	Sig.	CV (%)
1	Plant height	28.213	0.000^s	7.17
2	Leaf length	35.193	0.000^s	7.88
3	Leaft width	36.868	0.000^s	5.80
4	Stem length	10.257	0.000^s	11.86
5	Stem diameter	16.139	0.000^s	7.92
6	Number of tillers	7.696	0.000^s	18.61
7	Fresh sample weight	5.605	0.002^s	18.87
8	Total fresh weight	33.884	0.000^s	21.51

Initial Soil Analysis

Identification on the types and characteristics of the physical, chemical, and biological soil was intended to understand the level of soil fertility and ensure optimal plant productivity. Therefore, an initial soil analysis was carried out at the Soil Testing Laboratory (Table 2).

TABLE 2. Result of initial soil analysis of elephant grass mutant line trial plots in Cijeruk Highlands, Bogor.

Characteristics	Unit	Result	Rating*
pH H_2O	-	4.5	Sour
pH KCl	-	3.9	-
Nitrogen	%	0.14	Low
P_2O_5 HCl 25%	mg/100 g	38	Moderate
K_2O HCl 25%	mg/100 g	4	Very low
Cation-exchange capacity	cmol(+)/kg	12.13	Low

*Rating based on the Indonesian Soil Research Institute (Balittanah 2020).

Plant Height

Lines of M7 and M6 showed the highest plant height (226.86 cm), while the lowest plant height was obtained in M5 line (131.84 cm) (Table 3). According to Table 2, mutation induction treatment using gamma ray irradiation caused positive result, where two lines showed the increase in the plant weight exceeding the wild type. Nevertheless, there were several lines having a less more weight compared to their wild type. The plant weight was not controlled by many genes or polygenic; therefore, it was difficult to change. On the contrary, M5 line showed the lowest appearance of plant height, assumed due to a negative mutation value so that growth was stunted and not optimal. This corresponds with the statement of Soeranto [10] that rationally, mutations can cause changes in plant genetics in both positive and negative directions, and can also return to normal (recovery).

According to Sutapa and Kasmawan [11], the cells will be burdened by high kinetic energy due to the mutation from radiation, so that it affects chemical reactions of plant cells that changes chromosome of the plants. Gamma rays can suppress the growth of roots, stems and leaves. According to Makhziah *et al*. [12] that the gamma ray radiation dose of Co^{60} affects considerably towards the height character of plants and the number of leaves. The radiation effect can cause character degradation on the height of plants and the number of leaves. Apart from that, the growth of the M5 line treatment was not optimal because the growth of this line tended to widen and was more concentrated in the leaves. This line had a more prominent growth in leaf length and leaf width. This M5 mutant line had a fairly large stem but short stem segments, while the increase in plant height was directly proportional to the rate of stem elongation, so under these conditions the height growth was not optimal.

Leaf Length

The highest leaf length was obtained at 56 DAP in the lines of M6 and M7, while the lowest result was obtained in the M8 line. Those lines were not significantly different from control/wild type. M6 line had long and wide leaves, 101.11 and 3.33 cm, respectively (Table 3), so that the photosynthesis process took place effectively. In addition, the M6 line was considered to be able to adapt well towards growing environments, such as temperature and other factors in the highlands.

According to Hatta [13], the plant metabolic processes, such as photosynthesis, respiration, and photorespiration, are influenced by temperature. M8 line had the shortest leaf length, induction with gamma ray treatment can suppress plant vegetative growth, one of which is growth on leaves. According to Anshori [14], the induction of physical mutations with gamma ray irradiation causes different leaf shapes, including growth inhibition (dwarfism), leaf unification, and mosaic (color change).

Leaf Width

Lines of M6 and M7 showed the longest leaf width, but were not significantly different from the control. Meanwhile, the shortest average leaf width was obtained in M8 line. M6 line also showed the longest leaf length (Table 3), whereas M8 line showed the lowest leaf length.

TABLE 3. Plant height, leaf length, and leaf width of the elephant grass mutant lines planted in Cijeruk Highlands, Bogor at 60 days after planting.

Treatment	Plant height (cm)	Leaf length (cm)	Leat width (cm)
M0	216.71 a	96.84 a	3.32 a
M1	161.36 bc	59.56 c	2.27 bc
M2	153.45 bcd	58.62 c	2.38 b
M3	146.22 bcd	62.32 c	2.09 c
M4	165.03 bc	60.41 c	2.38 b
M5	131.84 d	74.03 b	2.53 b
M6	225.77 a	101.11 a	3.33 a
M7	**226.86 a**	100.54 a	3.32 a
M8	139.35 cd	52.41 c	2.08 c
CV	**7.17%**	**7.88%**	5**.80 %**

Numbers in one column followed by the same letter do not significantly different at the 5% level according to the DMRT.

Stem Length

The M7 line was not significantly different from lines of M6, M4, M1, and M0 in stem length, while the M5 line was significantly different from all treatments. M1 line was not significantly different from lines of M2, M3, M4, M6, and M8 (Table 4).

M7 line showed the highest stem length. This stem length growth was closely related to plant height parameters. It can be seen that the M7 line with the highest stem length was the line that obtained the highest on plant height parameters (Table 3). Genetic mutations can cause significant growth as well as slow growth. This was because mutations can cause random and sudden changes. This corresponds with the statement of Asadi [15] that the weakness of mutation breeding is random. Treatment of M5 line obtained the lowest stem length (59.23 cm) allegedly due to the results of genetic mutase causing slow growth. The effect of random mutation due to gamma ray irradiation caused physiological damage in the metabolism of cell development, so that the potential for growth can be faster or slower [14].

In addition, the growth of M5 line tended to be concentrated on the length and width of leaves and stem diameter. This M5 line had a fairly large leaf length, leaf width, and stem diameter. Soil fertility was assumed to have an effect on the growth rate of elephant grass stalks. Nitrogen (N) content in the trial plots was classified as low 0.14%. According to Daru *et al*. [16], nitrogen stimulates overall plant growth, especially stem growth because nitrogen plays an important role in the process of cell division.

Stem Diameter

The M7 line was not significantly different from M6 and control/wild type in stem diameter, these three lines were not significant on an average stem diameter, but those were significantly different from others (Table 4). The M7 line showed the highest stem diameter, the morphological character of this line had a large stem, so that its diameter growth was directly proportional to the size of the stem. Likewise, M8 line had the shortest stem diameter due to its small morphological character. In addition, it was suspected that a genetic mutation occurred as a result of gamma ray irradiation which caused the stalks of the line of M8 stunted. This is in accordance with Gea *et al*. [9], the morphological abnormalities such as small stem diameter and small leaves can occur as a result of irradiation dose.

TABLE 4. Stem length, stem diameter, and ratio of leaf length to stem length of the elephant grass mutant lines planted in Cijeruk Highlands, Bogor at 60 days after planting.

Treatment	Stem length (cm)	Stem diameter (cm)	Ratio of leaf and stem length
M0	139.94 a	2.06 ab	0.69
M1	126.77 abc	1.43 c	0.47
M2	109.02 bc	1.45 c	0.54
M3	110.16 bc	1.52 c	0.57
M4	129.70 abc	1.56 c	0.47
M5	59.23 d	1.87 b	1.25
M6	133.35 ab	2.05 ab	0.76
M7	142.91 a	2.13 a	0.70
M8	105.56 c	1.33 c	0.50
CV	**11.86%**	**7.92%**	

Numbers in one column followed by the same letter do not significantly different at the 5% level according to the DMRT.

Number of Tillers

The M3 line was not significantly different from M4 and M8 lines, but significantly different from other lines. The average number of tillers during the harvest period (60 DAP) with the highest yield was obtained in M3 line, while the lowest yield was obtained in M5 line (Table 5). Mutant lines that have small morphology, including the M1, M2, M3, M4, and M8 lines tended to produce a higher number of tillers compared to mutant lines that have large morphology line of M7, M6, M5 and M0/wild type. The M3 line had the highest yield because the growth of this line was more concentrated and produced a lot of tillers. This caused less growth on the height because nutrients in the soil were also used for the growth of the tillers in one clump. The M5 line that got the lowest results was due to genetic factors and soil fertility. According to Meliala *et al*. [17], the ability of plants to form tillers is influenced by the availability of nutrients and genetic factors of the plant.

Preliminary soil analysis (Table 2) showed that the soil in the trial field was less fertile with low N content (0.14%), low P (38 mg/100 g), and very low K (4 mg/100 g) causing low growth on the number of tillers. Acoording to Faizal *et al*. [18], the elements N, P, and K played a role in increasing the total tillers per clump. N element play a role in forming a pigment chlorophyll for photosynthesis, K element is for maintaining the activity of opening and closing of stomata associated with the acceptance of CO_2 for photosynthesis, while P element plays a role in increasing the work efficiency of chloroplasts and actively transferring very important energy within the cell in the process of cell division to form new offspring.

Fresh Sample Weight

The highest fresh sample weight was obtained from M6 and M7 lines, whereas the lowest was obtained from M1 line with the average of 0.57 kg. The M1 line was not significantly different from lines of M2, M3, M4, M5, and M8 (Table 5). The highest fresh sample weight (2.35 kg) shown by M6 line was assumed to be due to the genetic factor of the M6 line which had a large morphological size, so that a large morphological size would have a positive correlation with the yield of fresh weight and production at harvest. The large size of the morphology of the M6 line was argued to be the result of a positive genetic mutation that affected the growth of plant organs. This is consistent with the statement of Bermawie *et al*. [19] that gamma ray irradiation at low doses can cause diversity, such as developing leaves or changing shape, abnormal growth patterns, and can even increase plant organ size, production, and quality.

The small size of stem and leaf of M1 slowed down the rate of photosynthesis, and thus, inhibited its growth. Even though this line had the ability to produce quite a lot of tillers, the result of its small morphology caused low production. In addition, it was assumed that the M1 line had a negative genetic mutation and had low adaptability to environmental factors in the highlands, so that its growth was not optimal which caused low production.

Total Fresh Weight

The highest total fresh weight was obtained from the M6 line, while the lowest yield was obtained from M8 line. The line M6 was significantly different from all other lines. The result of M7 line were not much different from the control/wild type and not significantly different, but significantly different from other lines (Table 5). The M6 line which yielded the highest total fresh weight of 47.50 t/ha in this study was higher than that of Affandi [20] which stated that fresh production of elephant grass cv. Taiwan can be achieved by giving several packages of N, P, and K on yellow red Podzolic soil amounting to 31.80 t/ha per harvest. The yield of elephant grass production is influenced by various factors which affected its growth period during the study. Genetic and environmental factors, including soil, greatly influence plant growth, which in turn will determine production yields. Plant growth and production is strongly influenced by genetic factors of the plant itself, the better the plant species, the better its growth and production. Plant production is determined by the type of crop, climate, and management [21].

Genetically, it is argued that mutations have a positive value on the M6 line so that it has optimal growth and has the highest productivity compared to other mutant lines. In addition, this line is assumed to have the best adaptability among other mutant lines to environmental factors in the highlands during the study, this can be proven that the average M6 line always gets higher yields than the control for all the observed parameters. On the other hand, the treatment on the M8 line which obtained the lowest total weight on fresh sample was the result of a negative genetic mutation causing unpredictable instability in its growth, this is in accordance with the statement of Sutapa and Kasmawan [11] that breeding with physical mutations in gamma ray irradiation has several drawbacks; the acquired traits are unpredictable, and the instability of genetic traits that appear in the next generation is physiological damage due to genetic mutations that interfere with the metabolic process of the M8 line which causes stunted growth.

TABLE 5. Number of tillers, fresh sample weight, and total fresh weight of the elephant grass mutant lines planted in Cijeruk Highlands, Bogor at 60 days after planting.

Treatment	Number of tillers	Fresh sample weight (kg)	Fresh total weight (t/ha)
M0	17.89 cd	1.74 abc	37.95 b
M1	13.28 d	0.57 d	9,02 cd
M2	18.44 cd	0.86 d	10.53 cd
M3	28.78 a	0.99 cd	15.99 c
M4	26.11 ab	1.30 bcd	13.18cd
M5	12.22 d	0.91 cd	12.57 cd
M6	17.61 cd	2.35 a	47.50 a
M7	14.67 d	2.05 ab	38.89 b
M8	22.17 abc	0.61 d	6.99 d
CV	**18.61%**	**18.87%**	**21.51 %**

Numbers in one column followed by the same letter do not significantly different at the 5% level according to the DMRT.

Nutrient Content

The results of the proximate test analysis conducted at the Research Center for Bioresources and Biotechnology showed that the highest crude protein and crude fibers were obtained in the M7 line with crude protein (17.50%) and crude fibers (32.01%), while control obtained crude protein (15.08%) and crude fibers (28.86%) (Table 6). The M6 line obtained the lowest crude protein yield with a value of 14.00%. The M5 line obtained the lowest yield on crude fibers of 28.27%. The results of this nutrient content indicated the genetic diversity of each mutant strain of elephant grass that was induced by mutations, this was caused by random genetic mutations so that this genetic diversity can be generated.

TABLE 6. The nutrient content (%) of the elephant grass mutant lines planted in Cijeruk Highlands, Bogor as obtained from proximate test result.

Treatment	Crude protein	Crude fiber	Fat	Water content	Ash content
M0	15.08	28.86	2.65	16.45	7.67
M1	16.90	30.58	2.71	15.68	8.55
M2	14.84	29.92	2.48	16.38	8.86
M3	16.31	31.01	2.86	15.76	8.12
M4	15.23	30.56	2.18	17.27	7.47
M5	15.15	28.27	2.45	15.97	8.67
M6	14.00	29.96	2.89	16.79	6.97
M7	17.50	32.01	2.96	19.57	8.46
M8	16.89	30.35	4.57	18.83	6.69

Crude fiber content in elephant grass will affect the digestibility of feed in ruminants. The higher the crude fiber content, the lower the digestibility of the feed [22]. According to Rustiyana *et al*. [22], the crude fiber is negatively correlated with digestibility. The lower the crude fiber, the higher the digestibility of the ratio. The higher the crude fiber content in a feed ingredient, the lower the digestibility of the crude fiber will be. The crude protein content of elephant grass plants will decrease along with increasing plant age, this is in accordance with the opinion of Rustiyana *et al.* [22] that plant age is very influential on its nutrient content. Generally, the older the plant is at the time of cutting, the less protein content and the higher the crude fiber. Elephant grass at a young age has a better quality because of lower crude fiber but higher crude protein [23].

CONCLUSION

There were significant responses of the mutant lines of elephant grass (*P. purpureum* Schumach.) after gamma ray irradiation when planted in Cijeruk Highlands, Bogor, i.e. plant height, leaf length, leaf width, stem length, stem diameter, number of tillers, fresh sample weight, total fresh weight, and nutrient content. The M6 line had the highest yield on leaf length (101.11 cm), leaf width (3.33 cm), fresh sample weight (2.35 kg), and total fresh weight (47.50 t/ha). The M7 line had the highest yields on plant height (226.86 cm), stem length (142.91 cm), stem diameter (2.13 cm), crude protein content (17.69%), and crude fiber (32.48%).

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