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QUALITY OF ELEPHANT GRASS (*Pennisetum purpureum* Schumach.) GAMMA IRRADIATION RESULT IN THE SECOND GENERATION (MV2)

KUALITAS TANAMAN RUMPUT GAJAH (Pennisetum purpureum Schumach.*) HASIL IRADIASI GAMA PADA GENERASI KEDUA (MV2)*

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Abstract

Elephant grass (*Pennisetum purpureum* Schumach.) had crude protein 6.26%. Ruminant animals need 10–15% crude protein. An alternative way to increase the crude protein with induction by used gamma-ray irradiation. The purpose of this research were to determine the highest crude protein content and characterize accessions resulting from gamma irradiation for the selection process. The experimental design used a random block design. The first factor, gamma irradiation with level dose 0, 10, 20, 30, 40, and 50 Gy. The second factor, upper node, middle node, and lower node. The result were the highest crude protein occurred in the middle node with a dosage of 50 Gy with a value of 17.21% and a percentage increase 12.19%. The significant result occurred in agronomic characters of total of shoots in the second generation. The highest increase of crude protein occurred on the middle node dose of 50 Gy. Interaction of the upper node dose of 10 Gy was able to obtain the most shoots in the second generation. The increase in crude protein content and agronomic character of elephant grass was not stable because it was included in the early generation. The results of this research can be used as further research to obtain elephant grass with stable high crude protein content and stable elephant grass agronomic characters.

Kata kunci*:* Agronomic characters; Crude protein; Irradiation; *Pennisetum purpureum* Schumach

Abstrak

Rumput gajah (*Pennisetum purpureum* Schumach.) memiliki kadar protein kasar 6,26%. Ternak ruminansia membutuhkan protein kasar 10–15%. Cara alternatif untuk meningkatkan kadar protein kasar dan karakter agronomi dapat dilakukan dengan metode mutasi induksi menggunakan sinar gama. Penelitian ini bertujuan untuk mengetahui kadar protein kasar tertinggi dan melakukan karakterisasi aksesi hasil iradiasi gama untuk proses seleksi. Rancangan percobaan yang digunakan, yaitu rancangan acak kelompok faktorial. Faktor pertama adalah iradiasi dosis dengan taraf 0, 10, 20, 30, 40, dan 50 Gy. Faktor kedua adalah buku batang, yaitu buku batang atas, tengah, dan bawah. Hasil penelitian menunjukkan bahwa protein kasar tertinggi terjadi pada perlakuan buku tengah dengan dosis 50 Gy dengan nilai 17,21% dan peningkatan persentase sebesar 12,19%. Hasil berpengaruh nyata pada generasi kedua terjadi pada karakter agronomi jumlah tunas. Kadar protein kasar tertinggi terjadi pada buku tengah dosis 50 Gy. Perlakuan buku atas dosis 10 Gy memperoleh karakter jumlah tunas yang banyak pada generasi kedua. Peningkatan kadar protein kasar dan karakter agronomi rumput gajah yang diperoleh belum stabil, karena termasuk dalam generasi awal. Hasil penelitian ini dapat digunakan sebagai penelitian lanjutan untuk memperoleh tanaman rumput gajah dengan kadar protein kasar dan karakter agronomi rumput gajah yang stabil.

Keywords*:* Iradiasi; Karakter agronomi; *Pennisetum purpureum* Schumach.; Protein kasar

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INTRODUCTION

Elephant grass (*Pennisetum purpureum* Schumach.) is a type of forage that is widely used by breeders. Elephant grass has a nutrient content of 6.26% crude protein (Fathul et al., 2013). Crude protein plays an important role in the process of growth and development of livestock. Increasing the crude protein content of plants can be done by applying the induced mutation method through gamma ray irradiation. Induction of gamma rays entering the polypeptide chain encoded by nuclear genes in cells causes an increase in nitrate reductase activity (Alfariatna et al., 2018). The increased activity of nitrate reductase indicates that the energy used for nitrate reduction also increases and can provide a large capacity for the synthesis of amino acids, proteins, and total nitrogen (Umi, 2019).

Stem cuttings are one way of multiplying plants to produce uniform seeds. The selection of different stem parts will affect the speed of plant growth. Growth between stems is influenced by different levels of auxin and nitrogen from each part of the stem (Ramadan et al., 2014). According to the research of Hafiizh and Ermayanti (2014), plant breeding on elephant grass for shoot growth in vitro culture gave optimal results at a dose of 30 Gy. This is because the dose can maintain the viability of the shoots.

This research used the second generation of plants (MV2), namely the plant population produced from the first generation of vegetative propagation (MV1). MV1 is the first vegetatively propagated plant population (V1) of the generation mutant (M is defined as 'mutant') (Syarifah et al., 2009). The aims of the research were to determine the highest crude protein content in the MV2 generation elephant grass and to characterize the accessions resulting from gamma irradiation for the selection process.

MATERIALS AND METHODS

Tools and Materials

The tools used are the Multipurpose Panorama Irradiator (IRPASENA) with a radioactive cobalt-60 source, plant cuttings scissors, plastic tub with a size of 38 cm × 31.5 cm × 11 cm, digital microscope, leaf shears, oven, grinder, analytical balance, digester. OPSIS, distillator OPSIS liquidline, titrator titraline 5000, test tube, hot plate, Erlenmeyer, dropper, ruler, meter, stationery, label, and camera. The main ingredients used are elephant grass stems from the PAIR BATAN collection, ultisol soil, and manure. The chemicals used in the analysis of crude protein content were 0.02 N HCl, 97% H_2SO_4, 1 g selenium mix, 5% H_3BO_3 and 40% NaOH.

Experimental Design

The research used a randomized block design with 2 factors. The first factor is gamma irradiation which consists of 6 dose levels, namely D0 (0 Gy), D1 (10 Gy), D2 (20 Gy), D3 (30 Gy), D4 (40 Gy), and D5 (50 Gy). at a dose rate of 60 Gy/hour. The second factor is the 3 parts of the trunk of the elephant grass plant, namely the top (B1), middle (B2), and bottom (B3). The experimental unit was put into 3 groups as the number of replications. Each group has a spacing of 14 days. Individual plants used, i.e. all plants that survived in the first generation (MV1).

Research Procedure

Methode

The part of the elephant grass plant used consisted of the scion book, middle stem book, and rootstock book. The stem book is taken from the growing point of the plant. The rootstock book comes from the bottom 2 books, then the next 2 stem books are used as the middle stem book, and the next 2 books are used as the top stem book. The stems were planted 5 cm into the ultisol soil on a land measuring 15 x 15 m with a distance between plants of 30 cm.

Plants are maintained by watering 2 times a day, in the morning and evening. Weeds around the plants were weeded every day. The soil used is loosened 1 time in 1 week with the aim of distributing nutrients to the soil. Plants are fertilized using manure 1 time in 4 weeks as a nutrient in the plant growth process.

Observation and Data Analysis

Observations were made after 16 weeks after planting (MST) on the parameters of crude protein content and agronomic characters. The method used to determine the crude protein content is the Kjeldahl method. The sample used is from the second generation (MV2). Parts of the plant used, namely the whole leaves and young stems. The sample was then chopped using scissors to a size of 5 cm. The samples were mixed until homogeneous and baked at a temperature of 60 °C for 3 days. The dried sample was ground using a grinder, then 0.5 g was weighed and put into a test tube. The sample in the test tube was added with 1 g of selenium mix and 12 mL of H_2SO_4, then destroyed for 100 minutes at a temperature of 400 °C. The next step is distillation of the sample using 30 mL of 5% H_3BO_3 which has added methyl red and 50 mL of 40% NaOH solution. The final step is titrating the sample using a 0.02 N HCl solution. The end point of the titration is indicated by a color change to pink. The results of observations were calculated based on the formula (Cunniff, 1999), namely crude protein content (%)= $V\ x\ N\ x\ 14{,}008\ x\ 6{,}25$ divided by the mass of the sample (mg) multiplied by 100%. The value of V is the titration volume and N is the normality of the HCl solution. The formula for calculating the percentage increase in crude protein content is (A - B) divided by B multiplied by 100%. A is the rod interaction treatment with dose administration, while B is the rod interaction treatment without dose (control).

The parameters of the agronomic characters observed were (1) plant height (cm) measured using a meter starting from the soil surface to the highest leaf tip, (2) leaf length (cm) measured using a meter starting from the growing point of the leaf on the shoot to the leaf tip. the highest, (3) leaf width (cm) measured using a ruler in the center of the leaf from left to right of the leaf, (4) the number of leaves by counting the number of leaves that emerge from each shoot, and (5) the number of shoots by counting the number of shoots that appears from every plant book.

The measurement data were then analyzed statistically using ANOVA. If the results obtained were significantly different (P < 0.05), then continued with Duncan's test at a 5% significance level. The coefficient of diversity (KK) according to Susilawati (2015) is calculated using the formula $KK = \frac{\sqrt{KTG}}{\gamma ij} x\ 100\%.\ \gamma ij$ is the average of all treatments.

RESULTS

Levels and Percentage of Increase in Crude Protein of Elephant Grass in MV2

The dose irradiation treatment, stem book showed no significant effect (P>0.05) on crude protein content (Table 1). These results indicate that the dose irradiation treatment and plant stem books produced relatively the same protein content.

Table 1. Analysis of variance of crude protein content of elephant grass in MV2

SK	JK	DB	KT	F	Sig.
Group	451.08	2	225.54	82.46	0.00*
Dose (A)	16.89	5	3.38	1.24	0.31
Stem book (B)	0.65	2	0.32	0.12	0.89
(A) * (B)	20.22	10	2.02	0.74	0.68
Error	93.00	34	2.74		
Total	581.84	53			

Note: *: significant effect on the test F 5%

Table 2. Crude protein content of elephant grass in MV2 as a result of gamma irradiation

Treatment	Crude protein content (%)
B1D0	16.20
B1D1	14.51
B1D2	15.41
B1D3	15.87
B1D4	13.76
B1D5	15.68

Treatment	Crude protein content (%)
B2D0	15.34
B2D1	14.24
B2D2	14.41
B2D3	14.24
B2D4	14.60
B2D5	17.21
B3D0	15.77
B3D1	15.92
B3D2	15.37
B3D3	14.86
B3D4	14.53
B3D5	15.01
KK (%)	10.90

Note: B1 (top book); B2 (middle book); B3 (bottom book); D0 (0 Gy dose); D1 (10 Gy dose); D2 (20 Gy dose); D3 (30 Gy dose); D4 (dose of 40 Gy); D5 (50 Gy dose)

Table 3. The percentage increase in crude protein content of elephant grass in MV2

Treatment	Crude protein content (%)
B1D1	-10.43 ± 0.96
B1D2	-4.88 ± 0.96
B1D3	-2.04 ± 0.96
B1D4	-15.06 ± 0.96
B1D5	-3.21 ± 0.96
B2D1	-7.17 ± 0.96
B2D2	-6.06 ± 0.96
B2D3	-7.17 ± 0.96
B2D4	-4.82 ± 0.96
B2D5	12.19 ± 0.96
B3D1	0.95 ± 0.96
B3D2	-2.54 ± 0.96
B3D3	-5.77 ± 0.96
B3D4	-7.86 ± 0.96
B3D5	-4.82 ± 0.96

Note: B1 (top book); B2 (middle book); B3 (bottom book); D0 (0 Gy dose); D1 (10 Gy dose); D2 (20 Gy dose); D3 (30 Gy dose); D4 (dose of 40 Gy); D5 (50 Gy dose)

Table 4. Average agronomic character of elephant grass in MV2 as a result of gamma irradiation

Treatment	Plant height (cm)	Number of leaves	Number of shoots	Leaf length (cm)	Leaf width (cm)
B1D0	198.21	11.16	15.33^{abcd}	91.74	3.12
B1D1	184.28	17.31	39.67^{e}	90.22	3.07
B1D2	203.80	13.64	22.00^{cd}	100.77	3.38
B1D3	213.25	13.77	13.33^{abcd}	102.29	3.29
B1D4	217.99	11.53	19.00^{bcd}	101.39	3.37
B1D5	197.37	11.28	14.00^{abcd}	97.70	3.23
B2D0	212.83	12.07	12.00^{abcd}	104.43	3.16
B2D1	204.42	7.52	6.67^{ab}	103.52	3.02
B2D2	196.31	10.62	17.33^{abcd}	96.46	3.35
B2D3	177.82	14.54	8.67^{abc}	95.49	2.74
B2D4	192.51	10.46	16.50^{abcd}	99.46	2.81
B2D5	184.92	9.33	5.33^{a}	96.50	2.33

Treatment	Plant height (cm)	Number of leaves	Number of shoots	Leaf length (cm)	Leaf width (cm)
B3D0	183.26	11.99	21.00[cd]	98.34	2.88
B3D1	224.64	11.47	10.67[abcd]	107.19	3.43
B3D2	215.03	12.17	10.67[abcd]	106.46	3.13
B3D3	207.31	15.49	23.33[d]	93.18	3.05
B3D4	189.56	10.26	13.67[abcd]	91.68	3.17
B3D5	183.46	9.12	8.67[abc]	96.64	2.83
KK (%)	14.57	23.38	44.66	8.66	13.30

Note: The same superscript in the column shows no significant difference based on Duncan's test at level 5%; B1 (top book); B2 (middle book); B3 (bottom book); D0 (0 Gy dose); D1 (10 Gy dose); D2 (20 Gy dose); D3 (30 Gy dose); D4 (dose of 40 Gy); D5 (50 Gy dose)

Crude protein content of elephant grass with dose irradiation interaction treatment with trunk did not significantly affect this research ($P > 0.05$; Table 1). This means that the interaction treatment resulted in crude protein content with relatively the same value (Table 2). The interaction of irradiation dose and trunk showed an increase and decrease in percentage results compared to the control treatment (Table 3). The protein content that experienced the highest increase was treatment B2D5 and the treatment that experienced the lowest decrease was treatment B1D4.

Elephant Grass Agronomy Character in MV2

The interaction effect of dose irradiation and stem nodes did not show a significant effect ($P>0.05$) on plant height, number of leaves, leaf length, and leaf width (Appendix 1). The number of shoots parameter showed a significant effect with the highest number of shoots in treatment B1D1 (Table 4).

DISCUSSION

Levels and Percentage of Increase in Crude Protein of Elephant Grass in MV2

Crude protein content of elephant grass with dose irradiation treatment and stem book showed no significant effect ($P>0.05$; Table 1). This means that the given treatment produces protein levels with relatively the same value. The effect of radiation produced on MV2 has not affected the physiological process of elephant grass, so that genetically stable mutants have not been obtained. Stable mutants can be obtained because gamma rays can interfere with the process of protein synthesis, hormone balance, and enzyme activity (Suwarno et al., 2013).

Exposure to gamma ray irradiation at the given dose was not optimal to increase the crude protein content of elephant grass. Giving too high a dose will inhibit cell division (Hameed et al., 2008). According to Istanti et al. (2018), the optimal dose of irradiation treatment was able to increase the crude protein content of plants. This can be used to increase the nutritional value needed by ruminants. According to Handayati (2013), plant breeding in the early generation (MV2) genetically has not obtained stable mutants.

The dose treatment did not affect the crude protein content of elephant grass because there was a lot of water content (H_2O) in the plant. According to Herison et al. (2008), the more oxygen and water molecules (H_2O) in the irradiated material, the more free radicals will be formed. Free radicals generated from the radiation process can cause damage to plant DNA. Free radicals can damage or modify important components in plant cells so that some changes in morphology, anatomy, biochemistry, and plant physiology depend on the level of radiation (Gusti & Antha, 2016).

The percentage increase in protein content obtained random results (Table 3) because gamma irradiation was random (Sari et al., 2018). The negative sign in Table 3 means that the crude protein content has decreased. This means that the treatment interactions do not work synergistically in producing superior quality elephant grass. The B2D5 treatment (Table 3) showed an increase in the percentage compared to the control treatment. This treatment has the potential to become a superior elephant grass character when the next generation is passed down.

Bark did not significantly affect the crude protein content (P>0.05; Table 2). This is because the nitrogen content in each part of the stem is relatively the same. According to Wati et al. (2012), an increase in crude protein content in plants occurs along with an increase in nitrogen because nitrogen is used as a protein-forming agent. Mutations can occur as a result of changes in genes (Gusti & Antha, 2016). The information in a gene is determined by the sequence of nucleotides in the DNA. Genes give orders to form proteins through the process of transcription. Transcription produces mRNA, tRNA, and rRNA. The role of rRNA is in the assembly of ribosomes and tRNA carries the appropriate amino acids according to the information in the mRNA. The RNA gene is then combined with proteins as a protein synthesis process (Mustami, 2013).

The mid-book treatment with a dose of 50 Gy (B2D5) resulted in an increase in the highest percentage of total protein content of 12.19%. This indicates that the B2D5 treatment has the potential to produce stable high protein in the next generation. The percentage that decreased occurred in almost all treatments. Decreased interaction results are not used during the selection process.

Elephant Grass Agronomy Character in MV2

The dose of gamma irradiation exposed through the stems of the plant is not optimal so it does not affect the meristem cells in the stem. High doses of irradiation can inhibit plant growth because the enzymes that stimulate growth become inactive (Hafiizh & Ermayanti, 2014), while low doses of irradiation can generally maintain the viability of plant shoots (Soedjono, 2003). According to Parastiti et al. (2015), gamma irradiation can stimulate the growth of meristem cells at the tip of the stem by rapidly expanding to a certain extent so as to produce changes in plant height.

The dose irradiation showed a significant effect (P<0.05; Appendix 1) on the number of leaves. This shows that the effects of radiation exposed through the trunk only occur in the second generation. The highest number of leaves was found at a dose of 30 Gy. This means that a dose of 30 Gy is able to activate genes that affect cytokinin hormones for the formation of leaf organs. According to Devy and Sastra (2006), dose irradiation can change the balance of cytokinin hormones. The use of irradiation doses can activate genes to carry out the mRNA transcription process. Synthesis of mRNA from DNA has shown a coded pattern for the arrangement of amino acids that will form certain proteins through the translation process, so that they can synthesize growth hormone (Parastiti et al., 2015).

The interaction treatment of dose irradiation and stem nodes has not been able to change plant genetics in leaf organs because it is influenced by environmental factors, so dose irradiation interacted with stem nodes showed relatively the same number of leaves (Table 3). According to Suwarno et al. (2013), the speed of leaf emergence can be influenced by lighting, humidity, and environmental temperature. Plant maintenance carried out in a controlled manner causes the xylem to absorb the same nutrients so that the number of leaves produced is relatively the same.

The best interaction to produce the highest number of shoots was found in the B1D1 treatment (Table 3). This means that the irradiation dose of 10 Gy with the cusp is optimal for shoot growth. A dose of 10 Gy was able to change the arrangement of genes in the meristem tissue found in the scion nodes. Selection of the right dose can stimulate the endogenous auxin hormone. Auxins in plants play a role in the synthesis of DNA and RNA nucleotides and protein synthesis which are then used in the process of plant growth and development (Purba et al., 2017). Irradiation doses that are too high can inhibit shoot growth, because irradiation can cause shoot enzymes to be inhibited (Hameed et al., 2008).

The absorption of nitrogen content due to the radiation process gave relatively the same leaf growth. The element nitrogen plays a role in the formation of proteins. Radiation causes changes in the chemical composition of plant DNA so that it can change the synthesized protein. Changes in protein cause changes in metabolic processes in plant cells, resulting in changes in plant phenotypes (Syaifudin, 2005).

Radioactive light, if it hits plant tissue, will cause ionization of water molecules, then it will oxidize the sugars in DNA so that the nucleotide sequence will break. Radiation can cause nucleotide bases to become loose, damaged, or change their molecular structure. Radiation can also inhibit its replication and transcription and cause amino acids to not be produced because they are not read at the time of translation. Ionizing radiation absorbed into biological materials will react directly to critical cell targets or indirectly through the generation of metabolites that can modify important cell components (Sari et al., 2018).

Irradiation exposed through the stem did not change the DNA sequence in meristematic cells to stimulate the growth of leaf width. These results are in line with the research of Sutapa and Kasmawan (2016), which showed that irradiated tomato plants also had no significant effect on the character of tomato leaf width. Dosage to be able to obtain the character of the mutant plant depends on the type of plant, growth phase, size, and genetic material to be mutated. Plant species given exposure to gamma rays can give different responses because their sensitivity levels are also different (Rahman & Aisyah, 2018).

CONCLUSIONS AND SUGGESTIONS

The highest crude protein content in the MV2 generation occurred in the 50 Gy mid-book treatment with a value of 17.21% and the percentage increase of 12.19% compared to the control. The optimal 10 Gy dose of scion treatment obtained the most shoots in the MV2 generation. The increase in crude protein content and agronomic character of elephant grass is not stable because it is included in the early generation, so this research needs to be continued until crude protein content and stable agronomic characters are found.

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