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Articles

The Growth, Yield and Quality of Elephant Grass (*Pennisetum purpureum*) Specific Tolerant of Acid Soils by Mutagenesis with Ethyl methane Sulfonate

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Abstract. The purpose of this research was to know the effect of ethyl methane sulfonate (EMS) on the growth, yield and quality of Elephant grass. The experimental method with completely randomized design (CRD) was applied. Fifteen plot area of 2 x 3 m^2 were used in this experiment. There were three treatments and five replications. The treatments consisted of R0 = Elephant grass control, R1 = Elephant grass with 0.1% EMS treatment, and R2 = Elephant grass with 0.2% EMS treatment. The variables were the development (height of plant, the number of leaf, the leaf area and ratio of stem to leaf), yield (forage/plant, forage/plot and dry matter yield and quality (dry matter, crude protein and crude fiber content) of Elephant grass. The results of variance analysis showed that the treatments significantly affected the height of plant, the forage yield/plant and the crude protein content but did not significantly affected the number of leaf, the leaf area, ratio of stem to leaf, forage yield/plot, dry matter yield, dry matter and crude fiber content.

Keywords : ethylmethane sulfonate, *Pennisetum purpureum*, growth, yield, quality

Abstrak. Penelitian ini bertujuan mengetahui efek ethylmethane sulfonate (EMS) pada pertumbuhan, hasil dan kualitas rumput gajah. Metode eksperimen menggunakan rancangan acak lengkap. 15 bidang tanah berukuran 2x3 m^2 digunakan dalam eksperimen dengan tiga perlakuan dan lima pengulangan. Perlakuan terdiri dari R0 = rumput gajah kontrol, R1= rumput gajah dengan perlakuan 0.1% EMS, dan R2 = rumput gajah dengan perlakuan 0.2% EMS. Peubah terdiri dari perkembangan (tinggi tanaman, jumlah daun, luas daun dan rasio batang dan daun), hasil (panen/tanaman, panen/area tanah dan hasil kering dan kualitas hasil kering, protein kasar, dan serat kasar) rumput gajah. Hasil analisis variansi menunjukkan bahwa perlakuan sangat berpengaruh pada tinggi tanaman, hasil panen, dan kandungan protein kasar namun tidak berpengaruh besar pada jumlah daun, area daun, rasio batang dan daun, panen/area tanah, panen bahan kering dan kandungan protein kasar.

Kata kunci: ethylmethane sulfonate, *Pennisetum purpureum,* pertumbuhan, panen, kualitas

Introduction

Grasses in tropical areas are less digestible so that the consumption of nutrients that could be ingested by dairy cows is low (Raghuvansi et al., 2007). Provision of forage in marginal areas is still limited, so the farming of cattle is less developed, especially for large ruminants such as beef cattle and dairy cows. Acid soil is one of the lands found in marginal areas. Utilization of acid soil for cultivation as a source of forage as basal feed for ruminants requires specific grass that can grow and is tolerant to acid soils. Specific forage that can thrive in acid soils can be obtained through plant breeding. Some of the methods used for plant breeding are: introduction, selection, hybridization and genetic engineering approaches. This study emphasized the genetic engineering approach by way of mutagenesis. Mutagenesis is a mutation process. The plants that have mutations or that can show changes in phenotype due to mutation are called mutant, and the factors that cause mutations are called mutagens (mutagenic agents). Mutagenesis in plants can be induced by ethyl methane sulfonate (EMS (Parry et al., 2009). EMS has been known as a chemical mutagen that can alkylate thymine and guanine bases in such a way that changes the orientation of hydrogen bonds in both nucleotides. These changes result in changes in the replication of base pairs DNA molecules to where the GC is converted to AT and AT to GC. Therefore, mutations with EMS are included in the category of point mutations that occur randomly on bases of guanine and thymine (Akhmaloka et al., 2004). EMS can induce point mutations in the DNA molecule with a GC to AT transition (Dehkordi et al., 2008) and some plants that are mutated are more likely to have a phenotypic effect (Greene et al., 2003).

The function of EMS is made of groups from the AT to GC methylation, affecting environmental sustainability. The use of EMS has been widely used for instance in increasing root length in chili plants (Sri Devi and Mullainathan, 2011), increasing the amount of chlorophyll (Arulbalachandran and Mullainathan, 2009), and callus initiation (Luan et al., 2007).

Mutagenesis with EMS in Elephant grass will result in Elephant grass mutants. Mutant grass will result in differences in amino acids and methylation of AT to GC that affect resistance; one of which is the resistant to acid soil environments, so the grass is a source of forage mutant specific to location of acid soils. Therefore, it is necessary to evaluate the acid soils.

Materials and Methods

Materials

Materials used in this research were ethyl methane sulfonate (EMS), phosphate buffer, Elephant grass (*Pennisetum purpureum*) as a source of explants. Explants are 3cm stems of Elephant grass taken on 50 days old age.

Explants Preparation. The explants were cleaned off leaf sheaths and other debris with distilled water until they were clean. There were 3 explants soaking methods for 20 hours. The first treatment was soaking in distilled water of 15 explants (control), the second treatment with 0.1% EMS in phosphate buffer pH 6.68 (v/v) of 15 explants, and the third treatment with 0.2% EMS in phosphate buffer pH 6.68 (v/v) of 15 explants. Explants were grown on 2 x 5 m^2 land and waited up to 60-day old of age, and then they were cut and used as a source of stem cuttings.

Experimental Design

The treatments were arranged in a completely randomized design with three treatments (stick control Elephant grass, stick Elephant grass with 0.1% EMS treatment and stick Elephant grass with 0.2% EMS treatment). The treatments were repeated 5 times so that there were 15 experimental plots each of 2x3 m^2 size. Spacing cultivation was of 60 x 65 cm. The plants were cut at the age of 60 days.

Variables as indicators for the mutation were amino acid of leaves. Amino acid analysis used a High Petroleum Liquid Chromatography (HPLC), Shimadzu LC10 brand. Variables for growth included height of plant, number of leaves, leaf area, and the ratio of stems to leaves. Variables for the yields of grass were the yield of fresh grass per plant, total yield of fresh grass per plot and dry matter yield per plot. Variable for the quality included dry matter content of grass (DM), crude protein (CP) and crude fiber (CF) performed as directed by AOAC (2002).

Data for the analysis of amino acids was extracted from leaves of control Elephant grass which was composed of two samples of leaves and from Elephant grass that was treated with 0.2% EMS composited as many as four samples. Data of growth, yield and forage quality were obtained by sampling diagonally in each experimental plot.

Data analysis

Amino acid data were analyzed by t-test and data of growth, yield and forage quality were analyzed using analysis of variance, followed by Duncan test (Steel and Torrie, 1993).

Results and Discussion

Amino acid content of leaves as an indicator of mutation

The results of the analysis of amino acids of control Elephant grass and Elephant grass treated with 0.2% EMS (mutant Elephant grass) were listed in Table 1. Table 1 showed that there were 5 amino acids in the mutant Elephant grass that showed a significant difference compared to the amino acids in the control Elephant grass. This indicated that there was a change in the concentration of amino acids that was caused by EMS treatment. EMS was one of the reagents for the mutation that causes methylation of AT to GC.

Hierarchical cluster analysis

The plant number 1 and 2 were control Elephant grass, the plant number 3, 4, 5 and 6 were Elephant grass treated with 0.2% EMS. The analysis showed that amino acids in the dendrogram grouped the tested plant Elephant grass into 2. Plants 4, 5 and 6 that were treated in a 0.2% EMS joined as a group serving evidence of mutation, whereas mutant plants number 3 did not indicate mutants, despite treated with 0.2% EMS and joined the plant number 1 and 2 form a separate group included into group of Elephant grass (control). Hierarchy of amino acids is presented in Figure 1.

Growth of Elephant Grass

The results of measurements of plant height, number of leaves, leaf area, and the ratio of stems to leaves were listed in Table 2. Height of Elephant grass with 0.2% EMS treatment showed significant differences both with the control Elephant grass and with Elephant grass treated with 0.1% EMS. Height of Elephant grass treated with 0.2% EMS was higher than both the control Elephant grass and Elephant grass received 0.1% EMS treatment. This indicated that there was an increase in plant height, presumably because the mutation of genes in meristem tissue resulted in the increase in plant height. This was supported by Junita et al. (2002) that the increase in plant height are the result of the multiplication and extension of meristem tissue cells in the stem growing point. In a qualitative manner it can be described that the Elephant grass that received the 0.2% EMS treatment had a more powerful stem rigidity compared to control Elephant grass and Elephant grass receiving 0.1% EMS treatment, shown by the absence of collapsed plants when moderate winds blow. Another advantage of the Elephant grass with 0.2% EMS treatment was that the leaves were darker and greener.

Number of leaves, leaf area, stem and leaf ratio showed no significant difference between control Elephant grass with Elephant grass treated with 0.1% EMS and EMS 0.2%. This implied that there was the presence of gene mutations, however this did not lead to differences in those variables.

Forage yield of Elephant grass

The results of measurements of fresh forage yield and forage dry matter yield were listed in Table 3. Yield of fresh grass per plant showed significant differences between yield of fresh grass EMS 0.2% and 0.1% EMS treated or control grass. This implied that the yield of fresh grass per plant was higher, however the biomass was unable to show the differences. This was related to total yield of fresh grass per plot and yield of dry matter that showed no difference.

Table 1. Effect of EMS on amino acid content of leaves

Amino acids	Elephant grass control (ppm)	Elephant grass Mutan (ppm)	Significanty
Aspartic	20,343.22±503.03^{a}	18,900.79±175.73^{b}	0.024*
Glutamic	10,241.21±2586.03^{a}	9,973.99±963.07^{a}	0.281
Serin	1,164.02±33.72^{a}	856.52±108.06^{a}	0.133
Histidin	1,838.84±17.45^{a}	1,583.14±133.38^{a}	0.271
Glysin	3,242.50±70.07^{a}	1,294.96±658.88^{a}	0.120
Arginin	1,809.22±53.71^{a}	2,720.88±373.48^{a}	0.180
Alanin	221.18±133.63^{b}	2,130.73±541.25^{a}	0.079*
Tyrosin	2,690.07±8.19^{a}	1,415.50±391.69^{b}	0.096*
Metionin	18,494.23±33.17^{a}	19,095.62±273.68^{a}	0.217
Valin	224.42±187.41^{b}	1,812.10±348.73^{a}	0.041*
Phenil alanin	5,282.10±32.42^{a}	5,341.11±16.64^{a}	0.138
Ileusin	2,457.33±2.36^{b}	2,540.89±12.03^{a}	0.010*
Leusin	3,160.66±109.04^{a}	2,336.96±381.63^{a}	0.206
Lysin	663.05±122.67^{a}	838.77±117.96^{a}	0.412

Values bearing same superscripts at the same rows not significantly (P>0.10)

Table 2. EMS influence on plant height, leaf number, leaf area, the ratio of stem to leaf

	Control Elephant grass	0.1% EMS	0.2% EMS
Plant height (cm)	274.40 ± 14.81^{b}	278.00 ± 8.45^{b}	286.60 ± 9.74^{a}
Number of leaves (piece)	15.00 ± 0.55^{a}	15.80 ± 0.58^{a}	14.80 ± 0.49^{a}
Leaf area (cm^2)	2124.05 ± 11^{a}	3037.83 ± 44^{a}	2127.86 ± 46.73^{a}
Ratio of stem to leaf	3.39 ± 0.08^{a}	3.35 ± 0.11^{a}	3.23 ± 0.08^{a}

Values bearing different superscript at the same row differ significantly (P<0.05)

Table 3. Effect of EMS on the yield of fresh grass per plant and total yield of fresh grass per plot and yield of dry matter per plot

	Control Elephant grass	0.1% EMS	0.2% EMS
Yield of fresh grass per plan (g)	438 ± 52.09^{b}	458 ± 79.77^{b}	548 ± 33.82^{a}
Total yield of fresh grass per plot (kg)	51.03 ± 9.13^{a}	42.28 ± 4.85^{a}	49.35 ± 5.63^{a}
Yield of dry matter per plot (kg)	8.06±1.38^{a}	7.63±1.08^{a}	8.98±1.64^{a}

Values bearing different superscript at the same row differ significantly (P<0.05)

Table 4. Effect of EMS on dry matter, crude protein and crude fiber grass

	Control Elephant grass	0.1% EMS	0.2% EMS
Dry matter (%)	16.16±1.01^{a}	17.73±0.82^{a}	17.71±2.15^{a}
Crude Protein (%)	13.58 ± 0.07^{b}	14.81 ± 0.11^{a}	15.16 ± 0.16^{a}
Crude Fiber (%)	34.94 ± 0.16^{a}	34.03 ± 0.20^{a}	33.81 ± 0.19^{a}

Values bearing different superscript at the same row differ significantly (P<0.05)

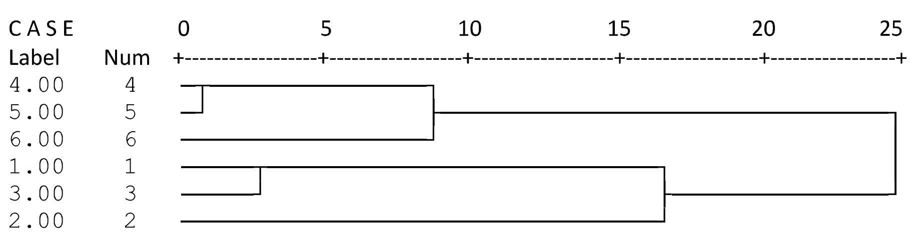

Figure 1. Amino acid dendrogram

Quality of forage

The results of chemical analysis of Elephant grass of dry matter content (DM), crude protein (CP) and crude fiber (CF) of Elephant grass experiment were listed in Table 4. Crude protein content of grass that was treated as well as 0.1% EMS and 0.2% EMS was significantly difference from control Elephant grass. This means that there was a mutation that was characterized by an increase in protein content which was a series of amino acids. Klungland et al. (1995) explains that the increase in AT of amino acid content was one of the characteristic of mutations in plants. The content of crude fiber and dry matter content of grass, however showed no significant difference.

Conclusions

The use of 0.2% ethyl methane sulfonate (EMS) can change control Elephant grass to Elephant mutant grass. The height of Elephant grass treated with 0.2% EMS is higher than both the control Elephant grass and Elephant grass receiving 0.1% EMS treatment. The yield of fresh grass per plant is higher, but the total yield of fresh grass per plot and yield of dry matter showed no difference. Crude protein content of grass treated with 0.1% EMS as well as 0.2% EMS is significantly different from control Elephant grass.

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