Performance of elephant grass BioGrass as in vitro breeding result in the highland of Bogor Regency, West Java, Indonesia

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Abstract. Low productivity of ruminants is mainly caused by the limited availability of high-quality forages as a result of reducing the area for pasture due to land conversion, lack of particular forage plantation, absence of forage breeder to control the quality, high rate of calf mortality, and low quality of forage varieties in the current period. Therefore, it is a necessity to generate a new superior variety of forages that are easily planted, adaptable to dry land, have high productivity, and high yielded. BioGrass as forage is a prospective strain of high yielding elephant grass varieties as a result of in vitro breeding that has high productivity and quality. This research aimed to determine the productivity and quality of BioGrass in the highland of Bogor Regency, West Java compared to local elephant grass and odot grass. The results showed that the speed of growth of bio grass cuttings buds was not significantly different from the speed at which local elephant grass buds and odot appeared. The bud colour of the leaf petals appeared reddish in bio grass while the local elephant grass and odot were green. The number of tillers and local elephant grass was 3-5 tillers while the number of odot tillers was 5-7 tillers. The fresh weight of bio grass and odot was 40 kg resulted from without fertilizing urea treatment and 60 kg with fertilizing urea treatment. Meanwhile, the fresh weight of local elephant grass was 60 and 90 kg, respectively based on urea treatments. Proximate analysis showed that the highest crude protein content (CP) of the BioGrass was 17.95% with the NDF and ADF value of 61.79 and 37.27%. Whereas CP of local elephant grass was 15.38% with NDF/ADF value of 63.15 and 39.01%, and CP of odot grass was 16.30 with NDF/ADF value of 33.64%.

1. Introduction
Feed plays the most important factor as a source of nutrients to increase livestock productivity, mainly cattle growth and reproduction. Forage is the main source of feed for ruminants since almost 90% of daily intake comes from forages as the amount of 10-15% of body weight. Other feeds are concentrated and supplements [1]. To achieve optimal productivity, feeding cattle must use good quality forages and meet the needs in terms of quantity, quality and continuity [2].

Elephant grass has been known for its benefits as ruminant feed in Southeast Asia [3]. Up to date, elephant grass (Pennisetum purpureum) developed in Indonesia consists of four cultivars, namely; 1) Pennisetum purpureum cv. Afrika Schumacher & Thons; 2) P. purpureum cv. Hawai Schumacher &
Thons; 3) *P. purpureum* cv. Taiwan Schumacher & Thons; and 4) *P. purpureum* cv. Moth. elephant grass cv. Africa and Hawaii were introduced to Indonesia in 1923, then cultivated by the Animal Husbandry Research Institute which is now the Livestock Research Institute (Balitnak) and disseminated to various locations in West Java in 1975 is the so-called first generation of Elephant grass in Indonesia. The second generation of elephant grass *P. purpureum* is cv. Taiwan and Moth were introduced to Indonesia in 2000 and have been distributed by Balitnak to various locations in West Java, Central Java, East Java, North Sumatra, Riau Islands, Bangka, and Kalimantan. Among the Elephant grass cultivars mentioned above, cv. Taiwan is the superior type of Elephant grass that is nowadays widely developed in Indonesia because it has high productivity and nutrition as well as palatability [4].

In general, the crude protein (CP) content of elephant grass is still low around 10.2%, whilst the other contents as follow: 1.6% fat, 34.2% crude fiber, 11.7% ash, 42.3% nitrogen-free extract, and 19.9% dry matter (DM) [5].

One common problem faced by traditional cattle farmers is low productivity due to low quality of forage and shortage of supply, especially during the dry season [6]. Low supply as the result of less land available for planting forages because mostly in the form of marginal land with low quality and have several limiting factors, such as dry land on ultisol soil types with low fertility levels. Therefore, technological innovation is required to improve productivity [7] and herb quality [8].

Technological innovation to obtain a new generation of elephant grass quickly and efficiently can be done by in vitro breeding using a combination of irradiation and in vitro selection. Husni et al [8] have succeeded in obtaining strains that have high potential in terms of productivity and herb quality; hence, these strains are used as genetic material in this study.

2. Methods

This study aimed to determine the productivity and quality of BioGrass in the highlands compared to local elephant grass and odot grass. The study consisted of 3 experiments, including germination in the greenhouses, plant performance in the highlands, and nutritional quality analysis for the herb. The research was conducted from August 2019 to January 2020.

2.1. Experiment 1. Germination in the greenhouse

The genetic material used was two-segment cuttings from three genotypes of mutant strains as a result of in vitro breeding (Strain R8/BioVitas, Strain R11/BioGrass, and Strain R16/BioNutris) and one local Elephant grass cv. Taiwan. The growth medium used was a mixture of soil and manure with a ratio of 3 using a polybag’s size of 15x20 cm and some 15 polybags per genotype. Maintenance was done by watering it using tap water every day. The variables observed at this stage were the number of shoots, shoot height, stems diameter and leaf length for 14 days.

2.2. Experiment 2. Plant performance in the highlands

The genetic material used was two-segment cuttings from two genotypes of Elephant grass, including mutant strains from in vitro breeding (BioGrass) and local elephant grass cv. Taiwan. The research method used was an experimental method using a completely randomized design single factor randomized model with 3 treatments (BioVitas, BioGrass and cv. Taiwan) and repeated 3 times so that there were 9 experimental units. Each strain treatment consisted of 30 clumps of plants so that there were 270 plants in total.

Soil processing was done by weeding the land. Furthermore, ploughing was carried out by hoeing to break the soil layer into chunks and turning the soil layer over and making holes with a depth of ±20 cm. Planting was done by inserting a stem cutting consisting of two segments, one book immersed in the soil and one book was above the soil surface. The cuttings were planted in an oblique position to the east, with a spacing of 100x75 cm.

The first fertilizer was spread one week before planting by applying manure at a dose of 10 tons/ha. The second fertilizer was carried out three weeks after planting, using 200 kg/ha of NPK fertilizer by immersing the fertilizer in the soil around each clump of elephant grass. Maintenance was carried out...
by weeding the grass in the research area in order not to interfere with plant growth. Harvesting was done when the elephant grass aged 60 days after planting by cutting the plants using a sickle with a cutting height of 5 cm from the ground.

2.3. Experiment 3. Analysis of the nutritional quality of the herb
The analysis was carried out on levels of CP, NDF and ADF only on BioGrass and local Taiwan strains and odot grass as a comparison. The analysis was done in the accredited Commercial Laboratory at the Livestock Research Institute in Ciawi based on [9] method using the IKM 02 test for protein analysis, IKM 09 for NDF, and IKM 10 for ADF.

3. Results and discussion
Forages, in term of grass and legume, have to be available sustainably both in quality and quantity. The availability of forage throughout the year varies depending on location, weather, season, soil quality and so on. Gea et al [10] reported that the growth in plant height and the number of mutant elephant grass leaves obtained from gamma-ray irradiation mutations showed a significant difference between observation after acclimatization in the greenhouse and the field. Therefore, in this study, the potential strains produced from in vitro breeding were tested to determine their genetics stability in the form of productivity and quality when cultivated in the highlands.

The analysis of variance from experiments one and two are shown in table 1. It can be seen that there were significant differences in the variables of shoot height, leaf length, shoot diameter, and several roots while the number of tillers was not significantly different. The significant different variables in experiment two were the number of tillers, stem diameter and stem length. The number of tillers and stem diameter were the most significantly different variables, while the stem length was just significantly different.

Table 1. Variance analysis matrix of observed variables based on the LSD test.

| Parameter | Genotype | $St_{0.05}$ (%) |
|-----------|----------|----------------|
| 1. Sprouts in the greenhouse | | |
| a. Number of shoots | ns | |
| b. Plant height | * | 16.5 |
| c. Shoot diameter | * | 12.0 |
| d. Leaf length | * | 9.8 |
| e. Shoot stem diameter | * | 14.5 |
| 2. Plant performance in the highlands | | |
| a. Number of tillers | ** | 5.61 |
| b. Plant height | ns | 9.11 |
| c. Stem diameter | ** | 4.33 |
| d. Stem length | * | 6.29 |
| e. Leaf length | ns | 7.82 |
| f. Leaf width | ns | 7.07 |
| g. Herb weight | ** | 4.98 |

Note: ns= non-significant; *significant using the LSD$_{0.05}$ **significant using the LSD$_{0.01}$

The growth of forage is described by an increase in size, shape, and amount as a result of physiological processes involving genetic and environmental factors. The main parameter of the growth which is often used as a superiority factor for a strain, clone, cultivar, and variety is plant weight that could determine the herb weight as the main part for feed. Other plant growth parameters also observed in this experiment were the number of shoots, plant height, number and width of leaves, stem diameter, and several roots. The analysis for these variables or parameters is shown in table 1 and 2.
Table 2 shows that the average number of genotype shoots of BioGrass strain was higher than that of BioVitas, BioNutris and local Taiwan strains although it was not significantly different. The average shoot height of the BioGrass strain was higher than the others. The average shoot height of BioGrass was 47.47 cm, followed by BioVitas, Local Taiwan, and BioNutris, which each was 27.63, 22.61 and 19.17 cm, respectively. The results of the experiment on shoot stem diameter variables were significantly different between genotypes. The shoot diameter of the BioGrass strain was wider (0.70 cm) than the BioVitas strain (0.54 cm) although it was not significantly different. However, the number was significantly different from the shoot diameter of BioNutris strain and local Taiwanese strain (0.34 and 0.37 cm, respectively). This shows that the potential for digestion weight of the BioGrass strain was higher than the other strains grown in the highlands.

![Table 2](image)

Table 2. The average number of shoots, shoot height, and stem diameter was 14 days after planting.

| Genotype          | Number of shoots | Shoots height (cm) | Shoot stem diameter (cm) |
|-------------------|------------------|--------------------|--------------------------|
| Galur R8 (BioVitas) | 1.47a            | 27.63b             | 0.54ab                   |
| Galur R11 (BioGrass) | 1.80a            | 47.47a             | 0.70a                    |
| Galur R16 (BioNutris) | 1.20a            | 19.17b             | 0.34b                    |
| Local (cv.Taiwan)  | 1.33a            | 22.61b             | 0.37b                    |

*ab* different superscript in the same column differ significantly (P<0.05)

Roots and leaves are very important organs for plants. Roots have generally functioned as nutrient absorbents for organic and inorganic materials that are needed for growth and development. Besides, the roots also have functioned as plant supports so that they can stand upright on the ground. While the main function of leaves is as a food processing plant through the photosynthesis process.

Table 3 shows that the average number of leaves of the genotype BioGrass strain was more and significantly different from the others. The average leaf length of BioGrass strain was 22.77 cm followed by BioVitas, local Taiwan, and BioNutris with lengths of 12.95, 11.87 and 7.07 cm, respectively. The average number of roots formed from the BioGrass strains was more than those from BioVitas and local Taiwan strains, although not significantly different.

![Table 3](image)

Table 3. The average number of leaves and the number of roots aged 14 days after planting.

| Genotype          | Number of leaf | Number of roots |
|-------------------|----------------|-----------------|
| Galur R8 (BioVitas) | 12.95b         | 0.33b           |
| Galur R11 (BioGrass) | 22.77a         | 2.13a           |
| Galur R16 (BioNutris) | 7.07b          | 1.20ab          |
| Local (cv.Taiwan)  | 11.87b         | 1.20ab          |

*ab* different superscript in the same column differ significantly (P<0.05)

The superiority of a strain from breeding can be obtained after modelling or testing in the field. The test results of the BioVitas and BioGrass strains in the highlands are shown in table 4 and table 5. From table 4, it can be seen that the BioGrass strains were superior to the BioVitas and local Taiwan strains. The number of tillers was 8.72, plant height was 158.87 cm, stem length was 64.5 cm, stem diameter was 2.10 cm, leaf length was 89.37 cm, and leaf width was 3.26 cm. Meanwhile, BioVitas in the same order were 7.68, 152.33 cm, 61.75 cm, 1.98 cm, 85.68 cm, and 3.01 cm and local Taiwan were 8.06, 154.20 cm, 64.79 cm, 1.85 cm, 84.27 cm, and 2.94 cm as the same order.
Table 4. The average number of tillers, plant height, and stem diameter at 14 days after planting.

| Genotype        | Number of shoots | Plant height (cm) | Stem length (cm) | Stem diameter (cm) | Leaf length (cm) | Leaf width (cm) |
|-----------------|------------------|-------------------|------------------|-------------------|-----------------|----------------|
| Galur R8 (BioVitas) | 7.68b            | 152.33as          | 61.75ab          | 1.98abc           | 85.68ns         | 3.01ns         |
| Galur R11 (BioGrass) | 8.72a            | 158.87            | 64.57a           | 2.10a             | 89.37           | 3.26           |
| Local (cv. Taiwan) | 8.06ab           | 154.20            | 64.79ab          | 1.85abc           | 84.27           | 2.94           |

abc different superscript in the same column differ significantly (P<0.05)

The main parameters of forages growth that is used as a superior determinant factor as cattle feed are productivity and herb nutritional values. The productivity of elephant grass is determined by the number of tillers, plant height, leaf length and width, stem length and stem diameter. The wet weight of the herb obtained from the test results in the highlands is shown in table 5. From this table, it can be seen that the BioGrass strain has the highest productivity and was significantly different from the BioVitas strain and Taiwan local with a herb weight of 15.48 kg/plot.

Table 5. Average leaf length, leaf width, herb weight and age productivity 60 days after planting.

| Genotype          | Herb weight (kg) | Productivity (ton/ha) |
|-------------------|------------------|-----------------------|
| Galur R8 (BioVitas) | 12.02bc          | 5.34                  |
| Galur R11 (BioGrass) | 15.48a           | 6.88                  |
| Local (cv. Taiwan) | 11.68c           | 5.19                  |

abc different superscript in the same column differ significantly (P<0.05)

Apart from the superiority of the herb weight produced from forages, the nutritional quality of the herb is also of great concern because 60–70% of all production costs are used for feed supply. The productivity and quality of forages grown in different climates affect the quantity and quality of herb due to differences in the length and intensity of sunlight [11]. Therefore, it is necessary to find substitutes for feed ingredients having the same nutritional value as commonly used feed. One effort to minimize production costs effectively is by feeding elephants grass because it does not compete with the interests of human consumption, besides, its availability is guaranteed throughout the year, highly nutritious, and has low production costs [12].

Table 6. Genotypes and levels of NDF and ADF of herb materials genotype of Elephant grass BioGrass, cv. Taiwan and Odot.

| Genotype                   | Protein (%) | NDF (%) | ADF (%) |
|----------------------------|-------------|---------|---------|
| Galur R11 (BioGrass)       | 17.95       | 61.79   | 37.27   |
| Local (cv. Taiwan)         | 15.38       | 63.15   | 39.01   |
| Local (Odot)               | 16.30       | 57.19   | 34.73   |

The test results on the protein content and digestibility of the NDF and ADF genotypes tested in this experiment are shown in table 6. It can be seen that the highest protein content was made up by BioGrass strains resulted from in vitro breeding with a value of 17.95% followed by each, local odot 16.30% and local Taiwan 15.38%. Based on the NDF and ADF values of the BioGrass elephant grass, the digestibility was classified as good because the NDF value was 61.79% and the ADF value was 37.27%. The NDF value of Biograss strains was lower than the local Taiwan NDF value and higher than that of
Thus, the BioGrass strains resulting from in vitro breeding is a new generation of elephant grass that can be developed in the highlands to provide high-quality forages for ruminants.

4. Conclusion

The genotypes of the mutant strains of BioGrass from in vitro breeding that was tested in this study showed to have a better performance than that of the existing local Elephant grass. The superiority can be seen from the length of leaves, plant height, stem diameter, and the number of roots in the germination experiment and the weight of herb from the performance experiments in the highlands, as well as the nutritional quality of protein content, NDF, and ADF.

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