Yield productivity test and morphological characterization of 19 sorghum lines resulted from mutation breeding

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Abstract. Sorghum has high potential and prospective to be cultivated and developed, along with increasing food and energy needs in Indonesia. Sorghum mutation breeding aimed to increase the variation, improve the yield and quality as food, raw materials bioethanol (energy) and animal feed. Yield productivity tests and morphological characterization play an important role in the preparation of adaptation tests as well as identifying character variations for the release of crop varieties. The research aims to test the yield productivity and characterize 19 sorghum mutant lines. The research was held from March to June 2016 at the Cibadak Research Station, BB-Biogen, Cipanas. The study used Augmented Design with 19 mutant lines (GHP-21, GHP-22, GHP-24, GH-25, GH-26, GH-27, GH-28, GH-29, GH-30, GH-31, GH-32, GH-33, GH-34, GH-35, GH-36, GH-37, GH-38, GH-39, and GH-40) and 3 national sorghum varieties as Control (Samurai-1, Samurai-2 and Super 2) as treatments. The observed parameters include plant height, number of leaves, stem diameter, time to flowering, panicle length, panicle weight, dry panicle weight, and a thousand seed weight. As for characterization, additional parameters were observed, including observations on the leaves, pistil, husk, panicle, and seeds. The results showed a noticeable difference in all the agronomic characters observed. There is GH-25 mutant line with the highest stem. The GH-40 line shows the earliest ripening. The GH-34 line saw the largest diameter. The GH-35 mutant line has the longest panicles. For the production observation parameters; panicles weight, dried panicles weight, and a thousand seeds weight, GH-33 line, GH-31 line, and GH-27 line have had the best results. Characterization results suggest that 19 sorghum-observed mutant lines have a sufficiently high degree of morphological similarity. The cluster analysis demonstrated by the level of similarity by 86%.

1. Introduction

Nowadays, the problem of food security and the energy crisis in Indonesia is still one of the main concerns. One of the alternatives to this problem is the inclusion of food diversification programs and the utilization of natural resources available and renewed as well as sorghum [1]. Sorghum can be used as the main crop and alternative food source to support food diversification. Sorghum can be used as a potential food to be grown and developed in Indonesia because of its good adaptation to the marginal land conditions. The potential of prone land area and temporary land not cultivated in Indonesia to reaching 8 million ha [2].
The amount of sorghum benefits is not accompanied by the socialization of excellence, utilization, and expansion of sorghum planting in Indonesia. Another obstacle to the sorghum development in Indonesia was sorghum is not a native Indonesian plant. The genetic diversity for the research program and superior varieties for the cultivation of sorghum in Indonesia are limited [3]. These constraints can be addressed by conducting plant breeding programs. One of them is the sorghum breeding program through the gamma-ray mutation techniques performed by BATAN.

The mutant lines resulted from mutation breeding still needs to be further researched regarding genetic diversity through morphological characterization, adaptability, and performance in the field. Therefore, multi-location trials and characterization test is required as the seed morphology marks the result of the mutation before it released as variety.

Characterization is an activity in germplasm to determine the morphological properties that can be utilized in distinguishing between accessions, assessing the amount of genetic diversity, identifying varieties and assessing the number of lines [4]. In addition, characterization aims to know and obtain data on excellence and its interaction with the environment of prospective varieties that will be released as superior varieties to be commercialized. The research aims to test the yield productivity and characterize 19 sorghum mutant lines.

2. Materials and Methods

The research was held from March to June 2016. The research site at Cibadak Experimental Station, Indonesian Centre for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRD), Cipanas, West Java which is at an altitude of ± 1130 m with the soil type of Andosol. This study used 22 sorghum genotypes as the genetic materials (19 mutant lines, two mutant varieties, one introduction varieties), goat manure, and chemical fertilizer. The tools which are used in this research were a hoe, water hose, ruler, rope, plastic, digital scales, stationery, and camera.

2.1. Yield Productivity Test

The study used Augmented Design with 19 mutant lines (GHP-21, GHP-22, GHP-24, GH-25, GH-26, GH-27, GH-28, GH-29, GH-30, GH-31, GH-32, GH-33, GH-34, GH-35, GH-36, GH-37, GH-38, GH-39, and GH-40) and 3 National varieties as Control (Samurai-1, Samurai-2 and Super 2) as treatments. The observed parameters include plant height, number of leaves, stem diameter, time to flowering, panicle length, panicle weight, dry panicle weight, and a thousand seed weight.

2.2. Morphological Characterization

The observed character refers to the guide of TG (Technical Guide)/122/4 for sorghum [5], with the number of 34 observation parameters. Observations were conducted using the scoring for each character of each line and comparative varieties. The observed characters include parts: leaves, pistils, husks, panicles, and seeds.

2.3. Data analysis

The data of yield productivity tests were subjected to a one-way analysis of variance (ANOVA) followed by mean separation by the Duncan Multiple Range Test (DMRT) test at P <0.05. Morphological Characterization data were processed with cluster analysis by R program to get an unsimilarity dendrogram.

3. Results and Discussion

Climatic data during this study got from the meteorological and geophysical agency in the Cipanas region. It suggested that in March 2016, the total rainfall is 607.5 mm, the average temperature is 21.7 °c, the sun's illumination intensity is 30%, and the average humidity is 87.6%. Subsequently, in April 2016, the total rainfall is 324.8 mm, the average temperature is 21.7 °c, the solar illumination intensity is 41.3%, and the average humidity is 83.1%. In May 2016, the total rainfall was 468.3 mm, the average temperature is 21.9 °c, the intensity of the solar illumination is 36.9%, and the average
humidity is 92.6%. While in June the total rainfall is 167 mm, the average temperature is 21.5 °C, the intensity of the solar illumination is 40%, and the average humidity is 77%.

During the study, the climate range is less in accordance with the requirements of growing sorghum plants that cause the inadequate and growth in the first 2 weeks to be hampered due to high rainfall and the intensity of the sun illumination. The inferior, nevertheless overall, nineteenth mutant sorghum lines and three comparative varieties undergo an excellent growth phase to harvest. The sorghum plant can grow and produce well in areas where the rainfall is high during the growth phase to harvest time [6].

| Genotypes   | Plant Height (cm) | Number of Leaves | Stem Diameter (cm) | Flowering Time (days after planting) |
|-------------|-------------------|------------------|--------------------|---------------------------------------|
| GH-21       | 122.79c           | 7                | 0.8e               | 76a                                   |
| GH-22       | 138.64bc          | 7                | 1.11cde            | 76a                                   |
| GH-24       | 199.20abc         | 7                | 1.01bc             | 68b                                   |
| GH-25       | 256.12a           | 7                | 0.75de             | 76a                                   |
| GH-26       | 172.14abc         | 8                | 0.97cde            | 76a                                   |
| GH-27       | 138.28bc          | 6                | 1.05cde            | 76a                                   |
| GH-28       | 187.68abc         | 10               | 1.44c              | 68b                                   |
| GH-29       | 173.61abc         | 7                | 1.37c              | 76a                                   |
| GH-30       | 185.28abc         | 7                | 1.45bc             | 76a                                   |
| GH-31       | 172.07abc         | 7                | 0.98cde            | 76a                                   |
| GH-32       | 146.90bc          | 5                | 1.33bc             | 68b                                   |
| GH-33       | 182.30abc         | 6                | 1.24c              | 76a                                   |
| GH-34       | 194.90abc         | 5                | 2.32a              | 76a                                   |
| GH-35       | 189.90abc         | 6                | 1.96ab             | 76a                                   |
| GH-36       | 163.49bc          | 6                | 1.21cd             | 64c                                   |
| GH-37       | 215.42ab          | 5                | 1.06cde            | 68b                                   |
| GH-38       | 172.33abc         | 7                | 1.56bc             | 68b                                   |
| GH-39       | 217.37ab          | 8                | 2.01cde            | 68b                                   |
| GH-40       | 210.20abc         | 8                | 1.7bc              | 64c                                   |
| Samurai 1   | 168.70abc         | 7                | 1.5c               | 68b                                   |
| Samurai 2   | 155.34bc          | 6                | 1.25c              | 68b                                   |
| Super 2     | 187.20abc         | 6                | 1.14cd             | 64c                                   |

Note: The numbers followed by the same letters in the same column are not significantly different based on DMRT tests at the 5% level.

3.1. Yield Productivity Test

3.1.1. Plant height. The observation results, the GH-25 line showed the highest of 256.12 cm is evident in the clear lines of GH-21, GH-22, GH-27, GH-32, GH-36 and Samurai 2 but did not differ markedly with other genotypes. The Agriculture Ministry of Agriculture Research and Development in Indonesia [7] stated that the sorghum plant height depends on the length and size of the stem. Sorghum has an average height of 2.6-4 m. The trees and sorghum leaves are similar to corn. High sorghum plants are closely related to the age and number of leaves, in high-maturity plants and fewer leaves than the aged plants. The height of the plant and the diameter of the trunk is an essential agronomic character as a criterion for the selection of sweet sorghum plants, it is related to total biomass. The higher plant and width of the stem are increasingly supported with the content of sugar (%brix), then the line is expected to be released into a superior variety [8].
3.1.2. **Stem diameter.** The GH-34 line (2.32 cm) showed the largest stem diameter. It was no different with GH-35, but it is different from the other lines, the difference in diameter of the stem on orghum lines is due to the genetic mutation factor. The diameter of the stem is a character associated with the ability of the sorghum rod in supporting the plant. A stem is a place of the rotation of nutrients absorbed by roots and photosynthesis from the leaves to all parts of the plant. Mutant lines that have higher stem biomass is expected to be used as raw material of the industry (bioethanol and fresh drinks). The stem biomass is needed as criteria in the sweet sorghum selection activity. In other words, the magnitude of the stem biomass in the mutant line will be able to produce a lot of stem juice in spacious units [9].

3.1.3. **Number of leaves.** The most number of leaves was GH-28 (10 strands). But it was not distinct from the other lines, suggesting that more and more leaves are the higher photosynthesis that occurs [10]. The number of leaves is closely related to the photosynthesis process. The more leaves in the plant are expected to be more and more photosynthesis that can be produced in the photosynthesis process.

3.1.4. **Time to Flowering.** The fastest-growing age of sorghum lines is GH-29, GH-30, GH-35, GH-37, GH-38, GH-39, GH-40, Samurai 1, Samurai 2 and Super 2 (64 DAP). The age of plants generally affects the production of seeds as well as biomass, because the age of plants relates to the length of the photosynthetic process period while photosynthesis is the primary producer of photosynthesis for plants, especially for the process of biomass formation [9]. Flowering occurs starting at the tip panicles than the bottom of panicle. The process of the flowers in one panicle took six to nine days. The rapid time of flowering age is influenced by short photoperiods and high temperatures.

3.1.5. **Panicle length.** The longest panicles were GH-35 line (37.43 cm) distinct with GH-22, GH-27, GH-32, and GH-39 but did not differ with other lines. Panicle size is determined by the number of fertile spikelets that are strongly influenced by the size of the plant and the rate of the distribution of dry materials during the formation of the panicle. The yield potential of sorghum seeds is determined by some result components that have a positive correlation with the character of the result such as panicle width, length of panicle, and the weight of panicle [11].

3.1.6. **Panicle Weight & a thousand seeds weight.** The most stringent wet panicle weight was the GH-33 line (166.05 g), distinct from GH-28, GH-30, GH-32, GH-34, GH-35, GH-36, GH-37, GH-38, Samurai 2 and Super 2 but did not differ with other lines. While the most robust dried panicles weight was the GH-31 line (142.77 g), it did not differ with GH-21, GH-22, GH-24, GH-25, GH-26, GH-27, GH-29, GH-28 and Super 2 but is different with other lines. The weight of a thousand most robust seeds is GH-27 line (42.81 g) not distinct from real with GH-21, GH-25, GH-26, GH-28, GH-29, GH-30, GH-31, GH-33, GH-35, GH-40 and Super 2 but is different from the other lines. Sungkono et al. (2009) state that the weight of the seed per panicle represents the accumulated growth and development of the generative phase and is the result per individual plant so that it becomes a significant character in the determination of seed yield.
Table 2. Data of panicle length (cm), panicle weight (g), dried panicle weight (g) and a thousand seed weight (g) of sorghum mutant lines in Cipanas, West Java

Note: The numbers followed by the same letters in the same column are not significantly different based on DMRT tests at the 5% level.

3.2. Morphological Characterization

3.2.1. Leaf Morphology. The results of observations on the leaves (Table 3) indicate that the sorghum breeding result of mutation has a wide variety of leaves, four lines have very narrow categories, three lines of narrow categories, five lines of wide category and eight lines along with three control varieties very wide group. Table 3 showed that on the observation of the leaves length there are three mutant lines (GH-24, GH-32, and GH-34) that have a short leaf, as well as seven mutant lines have longer leaves among the lines observed just like one control variety (Samurai-2). Genetic factors cause the long and wide character of the leaves. Xiao-Ping et al. [12] State the long-controlling gene of the leaf is located on chromosomes 6 and 10 and shows the effect of over-dominant. The wide-character controller gene is located on chromosomes No. 1, 6, and 4 with the dominant gene action, partially dominant and additive.

3.2.2. Flowering Time. Table 3 indicates that the mutant lines of the observed BATAN collection mutation have a varied flowering age, 9 of which are slow categories, six lines of medium and four lines of GH-27, GH-33, GH-36, GH-40 have a flowering time faster than two control varieties (Samurai-1, Samurai-2). Table 2 shows that nine sorghum mutant lines have a slow flowering age; it is likely due to low ambient temperatures that cause a stunted flowering phase. The flowering phase of sorghum is sensitive to high temperatures and low temperatures. High temperatures (40 °c) cause the occurrence of the emergence of panicles, steep reductions in crops, number of seeds, seed size, and Harvest index [13]. The low temperatures of 15 °c (noon) and 23 °c (night) can decrease the vigor of seeds, chlorophyll leaves, as well as the retards of flowering phases and filling panicles [11].
Table 3. Performance of leaf width (cm), panicle length (cm), and flowering time and their category of sorghum mutant lines in Cipanas, West Java

| Genotypes | Leaf Width (cm) | Category | Leaf Length (cm) | Category | Flowering Time | Category |
|-----------|----------------|----------|------------------|----------|----------------|----------|
| GH-21     | 8.9            | Wide     | 76.8             | Long     | 76a            | Slow     |
| GH-22     | 6.0            | Narrow   | 71.6             | Long     | 76a            | Slow     |
| GH-24     | 4.3            | Very narrow | 54.6           | Short    | 68b            | Medium   |
| GH-25     | 8.1            | Wide     | 69.2             | Medium   | 76a            | Slow     |
| GH-26     | 9.2            | Very Wide | 83.2             | Very long | 76a         | Slow     |
| GH-27     | 8.7            | Wide     | 82.4             | Very long | 76a         | Fast     |
| GH-28     | 9.8            | Very Wide | 81.3             | Very long | 68b         | Medium   |
| GH-29     | 9.4            | Very Wide | 90.6             | Very long | 76a         | Slow     |
| GH-30     | 9.1            | Very Wide | 98.2             | Very long | 76a         | Slow     |
| GH-31     | 6.3            | Narrow   | 67.5             | Medium   | 76a            | Slow     |
| GH-32     | 4.5            | Very narrow | 52.4           | Short    | 68b            | Medium   |
| GH-33     | 8.6            | Wide     | 73.4             | Long     | 76a            | Fast     |
| GH-34     | 5.8            | Very narrow | 57.1           | Short    | 76a            | Slow     |
| GH-35     | 6.7            | Narrow   | 70               | Medium   | 76a            | Slow     |
| GH-36     | 8.9            | Wide     | 72.5             | Long     | 64c            | Fast     |
| GH-37     | 9.4            | Very Wide | 87.2             | Very long | 68b         | Medium   |
| GH-38     | 9.1            | Very Wide | 70.6             | Long     | 68b            | Medium   |
| GH-39     | 9.7            | Very Wide | 81.1             | Very long | 68b         | Medium   |
| GH-40     | 9.3            | Very Wide | 73.5             | Long     | 64c            | Fast     |
| Samurai-1 | 9.7            | Very Wide | 75.8             | Long     | 68b            | Medium   |
| Samurai-2 | 9.5            | Very Wide | 83.4             | Very long | 68b         | Medium   |
| Super-2   | 9.5            | Very Wide | 78.3             | Long     | 64c            | Fast     |

Description: Panicle Width: very narrow = < 6 cm, narrow = 6.1-7 cm, medium = 7.1-8 cm, area = 8.1-9 cm, very wide = 9.1-10 cm. Panicle Length: Very short = < 50 cm, short = 50.1-60 cm, medium = 60.1-70 cm, length = 70.1-80 cm, very long = > 80 cm. Flowering Time: Fast = 56-65 days, moderate = 66-75 days, slow = 76-85 hari, very slow = > 85 days.

3.2.3. Panicle Morphology. The medium and long category panicle neck is a wanted character of sorghum because the medium and long neck panicles ideally facilitate the time of harvest [14]. The results of observations on the leaves (Table 4) indicate that the mutation-induced a wide variety of leaves, four lines have very narrow categories, three lines of narrow categories, five lines of wide category and eight lines along with three control varieties very wide group. The panicle shape of sorghum-based on the UPOV [5] description consists of five criteria: Inverted pyramid, wider upper part, wide middle part, wide bottom an, and a pyramid. The observation result (table 4) indicates that the three lines have a wider upper part, ten lines have a wider middle part, and six have the lower part of the panicles of the section (Broad lower part).
Table 4. Performance of panicle neck length (cm), panicle shape, husk, and seed colour and their category of sorghum mutant lines in cipanas, west java

| Genotypes  | Panicle Neck Length (cm) | Category | Panicle Shape               | Husk Colour    | Seed Colour |
|------------|--------------------------|----------|-----------------------------|----------------|-------------|
| GH-21      | 22                       | Very long| broad middle part           | Yellow         | Yellow Straw|
| GH-22      | 23.2                     | Very long| broad middle part           | Yellowish Red  | Yellow Straw|
| GH-24      | 23.5                     | Very long| broad middle part           | Reddish Brown | Yellow Straw|
| GH-25      | 17                       | Long     | broad lower part            | Reddish Brown | Grey White  |
| GH-26      | 22.4                     | Very long| broad upper part            | Yellow         | Grey White  |
| GH-27      | 23.3                     | Very long| broad middle part           | Yellow         | Yellowish white|
| GH-28      | 24.3                     | Very long| broad lower part            | Yellow         | Grey White  |
| GH-29      | 16.7                     | Long     | broad lower part            | Yellow         | Reddish Brown|
| GH-30      | 21                       | Very long| broad lower part            | Yellow         | Dark brown  |
| GH-31      | 16.5                     | Long     | broad middle part           | Yellow         | Yellow Straw|
| GH-32      | 21.6                     | Very long| broad middle part           | Reddish Brown | Light brown |
| GH-33      | 20.4                     | Very long| broad upper part            | Yellow         | Yellow Straw|
| GH-34      | 20.8                     | Very long| broad middle part           | Yellow         | Yellow Straw|
| GH-35      | 22.2                     | Very long| broad middle part           | Yellow         | Yellow Straw|
| GH-36      | 15.8                     | Long     | broad middle part           | Yellow         | Grey White  |
| GH-37      | 19.4                     | Long     | broad upper part            | Yellow         | Yellowish white|
| GH-38      | 18                       | Long     | broad lower part            | Yellow         | Yellowish white|
| GH-39      | 17                       | Long     | broad middle part           | Yellow         | White       |
| GH-40      | 21.6                     | Very long| broad lower part            | Yellow         | Yellowish white|
| Samurai-1  | 17.5                     | Long     | broad lower part            | Yellow         | Yellowish white|
| Samurai-2  | 16                       | Long     | broad middle part           | Yellow         | White       |
| Super-2    | 22.5                     | Very long| broad middle part           | Yellow         | Grey White  |

Description: Panicle Neck Length: very short = < 5 cm, short = 5.1-10 cm, medium = 10.1-15 cm, long = 15.1-20 cm, very long = > 20 cm.

3.2.4. Husk and Seed Morphology. Colour husk sorghum based on the description UPOV [4] consists of white, yellowish-white, yellow, whitish gray, reddish-brown, blackish-brown, and black. Sorghum's line of mutation has a yellowish-white, yellow, reddish-brown husk (Table 4). Sedgi et al. [15] state that the sorghum that has light-coloured husk is more well-liked for foodstuffs than the sorghum with a dark-coloured husk. Sorghum with dark seeds (brown, reddish-brown) is more resistant to fungi because it has a higher phenol content, while the sorghum with bright seeds (white, yellow) is usually used for food [15]. The sorghum mutant lines have a diversity of seed colour characters, namely white, yellowish-white, straw yellow, grayish-white, light brown, reddish-brown, and dark brown (Table 4).

3.2.5. Genetic Distance of Sorghum Mutant Lines. Similarities between tested species can be viewed using Cluster Analysis (group analysis) which is a phylogenetic or Dendrogram [16] grouping of 19 sorghum mutant lines. Based on Figure 1 it is possible that the highest degree of similarity for 22 genotypes is 86% could be grouped into two groups. In the first group of GH-38 with a K-2 percentage of similarity 99%, GH-39 with K-4 has a percentage of similarity 98%, it showed that GH-38 with K-2, GH-39 with K-4 had a very close genetic distance and showed same morphological character. The GH-27, GH-33, GH-36, GH-37, GH-40 lines have a genetic distance that is adjacent to the similarities 94-95% degree of the percentage and shows the appearance of the morphological character not much different, GH-31 has a percentage of similarity 89.5% against other sorghum mutant lines within the group.
In the second group of GH-29 with GH-30, GH-26 with GH-28, GH-24 with GH-34, GH-21 with GH-35 has an adjacent genetic distance based on its morphological appearance. The GH-25 and GH-32 lines had a percentage similarity of 9% of the observed mutant sorghum lines. GH-22 has a 6% percentage similarity of the observed sorghum mutant lines within the group.

![Cluster Dendrogram](image)

**Figure 1.** Dendrogram based on into morphological characters 19 mutant lines of sorghum and three control varieties

Nineteen sorghum mutant lines can be said to have a resemblance of morphological character high enough because it is only able to demonstrate the level of similarity testing by 86%. It is suspected that all planting materials before in radiation by gamma rays are derived from one of the same elders, it will slap the morphology, which is not much different. In general, it can be said that the higher the level of the resemblance, the lower the diversity of the genetic and genotyping arrangement is suspected to be uniform.

4. **Conclusion**
- GH-33, GH-34, and GH-35 lines have better results than Samurai-1, Samurai-2, and Super-2 control on the observation parameters of stem diameter, dry panicle weight, the weight of a thousand seeds.
- Production observation parameters; panicles weight, dried panicles weight and a thousand seed weight for the genotype of GH-25, GH-26, GH-27, GH-28, GH-29, GH-30, GH-31, and GH-32 get the most excellent results.
- Based on the morphological apparition at a similarity level of 86%, 22 sorghum mutant strains can be grouped into 2 groups. The first group consisted of nine sorghum mutant lines and 3 control varieties, namely GH-31, GH-27, GH-40, GH-36, GH-33, GH-37, GH-38, GH-39, Samurai 1, Samurai 2, Super 2. The second group consisted of eleven sorghum mutant lines, namely GH-29, GH-30, GH-25, GH-26, GH-28, GH-32, GH-24, GH-34, GH-22, GH-21, GH-35.

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