Agronomic characters evaluation of sweet sorghum bicolor (l.) moench mutants

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Abstract. Sorghum is an annual crop with a lot of varieties. Recently, sweet sorghum is recognized as a potential new generation bioenergy crop because of its potential to accumulate sugar (10-15%) in its stalk similar to sugarcane, apart from producing grains. This study aims to evaluate agronomic characters including sugar contents of M3 and M4 population of sweet sorghum mutants derived from Numbu variety. The mutants were produced by combining in-vitro culture with induced mutation using gamma ray irradiation. The study was conducted at ICABIOGRAD Experiment Station (KP Cikeumeuh) in Bogor-Indonesia from July 2015 to June 2016. The study was arranged in augmented, the selection of selected lines for the next test material was based on agronomic characteristics of plant height, stem diameter and sugar Brix, which were superior to its parents, namely Numbu. The selection of selected lines for the next test material is based on agronomic characteristics of plant height, stem diameter and sugar Brix, which is superior to its parents, namely Numbu. Evaluation and selection in M3 and M4 population were focused on important agronomic characters such as plant height, stem diameter, stem sugar content, panicle length and weight, and yield/plant. The results indicated that 13 mutants having higher plant, bigger stem diameter, higher Brix value, seed production and higher yield/plant than their parent, Numbu. Have been selected from M3 and M4 population the selected mutants (M5) will be used further for yield trials.

1. Introduction
Sorghum includes high productivity C4 cereals which are potentially developed in Indonesia because they are able to adapt to marginal land such as acid and dry land [1]. Sorghum with sugar content in the stem > 8 Brix referred to as sweet sorghum. Sugar content in sweet sorghum stems rom fructose fraction, sucrose and glucose [2]. Therefore, sweet sorghum can be regarded as a multipurpose plant because in addition to the seeds can be for food, also juice from stalk and starch of the seeds have the potential as raw materials to be processed into ethanol or ethyl-butyl ester as an octane additive for gasoline, while the bagasse can be used as materials animal feed [3].

Sorghum is not native to Indonesia, so its genetic variety is limited. There are only two new superior varieties (VUB) of sweet sorghum released, namely Super 1 and Super 2. Super 1 was developed from a selection of local pure strains of Water Hammu Putih from Sumba, NTT and Super 2 originated from the introduction of the ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) breeding line (http://pangan.litbang.pertanian.go.id). Therefore, the extension of genetic varieties must be done so that the selected genetic material can be selected to regenerate the sweet sorghum VUB [4]. One method that can be used to increase plant genetic diversity is through mutations both physically and chemically [5], [6]. A combination of physical mutations with gamma-
ray irradiation and in vitro cultures has been performed to obtain fungus-resistant feather bananas [7] and progeny of Fatmawati rice varieties that are more resistant to blast disease [8].

From the previous research has been obtained a sweet sorghum mutant population, derived sorghum Numbu varieties, produced through a combination of gamma-ray irradiation with in vitro culture. Selection activities are an important stage in plant breeding programs and their success depends on the individual genetic variation of the selected population. The purpose of this research is to obtain mutant strains of sweet sorghum M₄ and M₅ which have good agronomic characters including seed yield and sugar content with high Brix value.

2. Materials and Methods

The experiment was conducted at Cikeumeuh Experiment Garden, BB Biogen Bogor from July 2015-June 2016. The experiment consisted of two activities, namely: [1] Evaluation of agronomic character and yield potential of 100 M₃ strains, [2] Evaluation of agronomic character and yield potential of 40 M₄ strains.

2.1. Evaluation of agronomic character and yield potential of 100 M₃ strains

The genetic material tested was 100 M₃ mutant strains, each line grown in a row, 2.5 m long lines without replicates consisting of 10 holes and each hole planted two seeds, with a spacing of 75 x 25 cm. The first fertilization was done on 12 days after planting plants with a dose of 100 kg urea and 150 kg of Ponska and subsequent fertilization when the plant was 35-40 days after planting with a dose of 150 kg urea and 150 kg of Ponska/ha. The experimental design was augmented every 25 numbers planted by the parents Numbu. The variables observed in the five sample plants were [1] plant height measured from the base of the stem to the exit of the panicle, [2] stem diameters (bottom, middle and top), [3] panicle length, [4] wet and dry weight of panicle, and [5] Brix value of stem sugar juice.

2.2. Evaluation of agronomic character and yield potential of 40 M₄ strains

The genetic material for potential yield was 40 mutant strains of M₄, each strain planted in a plot of 3 m x 2.5 m. It consists of 4 rows with spacing between lines 75 cm and in rows 25 cm. Each row consists of 10 holes, one hole was planted with 2 seed. So, the number of each strain planted was 80 seeds.

In the experiment, the land processing is done until the soil condition is loose. The first fertilization was done on 12 days after planting plants with a dose of 100 kg urea and 150 kg of Ponska and subsequent fertilization when the plant was 35-40 day after planting with a dose of 150 kg urea and 150 kg of Ponska/ha. The variables observed in the five sample plants were [1] plant height measured from the base of the stem to the exit of the panicle, [2] stem diameter (bottom, middle and top), [3] panicle length, [4] wet and dry weight of panicle, and [5] Brix value of stem sugar juice.

3. Results and Discussion

3.1. Selection on M₃ mutan

Mutant strains in the M₂ and M₃ generations were selected using the pedigree method, plants with superior characters such as plant height, stem diameter, panicle weight and sugar Brix higher than their parents were selected for further testing. The diversity of characters in the tested population provides an opportunity to obtain new varieties that are expected, according to [9] research, on the assembly of sorghum varieties. Yield potential test of hope lines of sorghum mutan by [10] showed the result of diversity in agronomic character and yield component.

The weight of a dried panicle is important in determining the high yield strains. Among 298 plants observed, 30 mutans have higher wet panicle weigh compared to the parent, about 92 g - 96.7 g/plant, whereas in the 67 g (Table 1). As a good raw material, it is necessary to develop varieties that have a high yield and nutritional quality. As for bioenergy (bioethanol), cultivars need to be planted high seed yield, good seed quality, and high sugar content [10].
| Number of lines | Plant height (Cm) | Stem Diameter (cm) | Brix value (%) | Length of Panicle (cm) | Weight of wet panicle (g) | Weight of dry panicle (g) |
|-----------------|------------------|-------------------|---------------|-----------------------|--------------------------|--------------------------|
| N.40.1          | 316.1            | 1.8               | 14.0          | 21.8                  | 49.3                     | 26.4                     |
| N.40.2          | 317              | 2.0               | 13.0          | 22.7                  | 57.5                     | 43.0                     |
| N.40.3          | 287.7            | 1.8               | 11.0          | 21.0                  | 73.5                     | 61.1                     |
| N.40.4          | 314.2            | 1.8               | 12.4          | 22.2                  | 40.5                     | 30.1                     |
| N.40.5          | 279.3            | 1.9               | 12.1          | 20.0                  | 57.0                     | 46.3                     |
| N.40.6          | 311.5            | 1.9               | 11.7          | 21.5                  | 58.6                     | 42.5                     |
| N.40.8          | 325.7            | 1.7               | 11.5          | 21.0                  | 55.2                     | 41.4                     |
| N.40.9          | 300.3            | 1.6               | 10.8          | 21.3                  | 76.5                     | 56.2                     |
| N.40.10         | 327              | 1.6               | 14.7          | 21.1                  | 83.7                     | 41.3                     |
| N.40.11         | 292.3            | 1.6               | 13             | 18.9                  | 31                       | 25.3                     |
| N.40.12         | 303.3            | 1.3               | 13             | 18.9                  | 31                       | 25.2                     |
| N.40.13         | 301              | 1.7               | 10.7          | 20.0                  | 54.2                     | 44.4                     |
| N.40.14         | 308.7            | 1.4               | 11             | 20.8                  | 44.8                     | 37.9                     |
| N.40.16         | 258.3            | 1.3               | 10.1          | 20.7                  | 46.7                     | 38.9                     |
| N.40.17         | 313.2            | 1.4               | 9.5           | 20.4                  | 42.8                     | 34.3                     |
| N.40.19         | 289.6            | 1.6               | 12.4          | 20.6                  | 47.9                     | 35.6                     |
| N.40.20         | 312.8            | 1.6               | 13.5          | 20.7                  | 49.3                     | 39.7                     |
| N.40.21         | 331.3            | 1.6               | 12.7          | 20.9                  | 46                       | 38.6                     |
| N.40.24         | 301              | 1.4               | 9.7           | 20.8                  | 32.2                     | 24.6                     |
| N.40.25         | 307              | 1.4               | 12.2          | 21.0                  | 39.2                     | 32.9                     |
| N.40.27         | 295.8            | 1.5               | 12.3          | 20.8                  | 47.8                     | 32.6                     |
| N.40.28         | 315.8            | 1.5               | 12.1          | 18.7                  | 43.3                     | 34.6                     |
| N.40.30         | 309              | 1.6               | 13.3          | 18.8                  | 27.7                     | 21.2                     |
| N.50.37         | 269.3            | 1.5               | 10.3          | 21.3                  | 54.4                     | 41.5                     |
| N.50.38         | 272              | 1.5               | 11.1          | 21.0                  | 47.9                     | 40.3                     |
| N.50.39         | 327.2            | 1.6               | 10.9          | 20.3                  | 53.8                     | 43.4                     |
| N.50.40         | 302.5            | 1.3               | 13             | 20.2                  | 46.5                     | 41.4                     |
| N.50.41         | 304.0            | 1.7               | 11.8          | 21.2                  | 48.3                     | 39.9                     |
| N.50.42         | 288.3            | 1.4               | 10.3          | 19.3                  | 46.8                     | 37.9                     |
| N.50.43         | 292.7            | 1.4               | 11.3          | 20.2                  | 28.6                     | 19                       |
| N.50.44         | 263.7            | 1.5               | 11.2          | 20.2                  | 45.7                     | 35.4                     |
| N.50.46         | 289.6            | 1.5               | 12.3          | 18.8                  | 41.2                     | 34.2                     |
| N.50.48         | 289.6            | 1.4               | 10.5          | 20.6                  | 64.4                     | 52.7                     |
| N.60.50         | 289.6            | 1.4               | 13.6          | 20.3                  | 58.2                     | 50.5                     |
| N.60.51         | 316.6            | 1.6               | 12.8          | 19.9                  | 39.1                     | 30.9                     |
| N.60.52         | 332.6            | 1.5               | 13             | 20.1                  | 52.9                     | 39                       |
| N.60.53         | 307.4            | 1.5               | 13.6          | 21.9                  | 48.2                     | 34.0                     |
| N.60.54         | 300.6            | 1.8               | 10.5          | 20.7                  | 54.8                     | 44.5                     |
| N.60.55         | 336.6            | 1.6               | 11.3          | 21.5                  | 57.1                     | 44.5                     |
| N.60.57         | 300              | 1.5               | 9.1           | 20.2                  | 68.5                     | 56.3                     |
| N.60.58         | 289.3            | 1.6               | 11.3          | 21.5                  | 69.2                     | 59.5                     |
| N.60.59         | 294.6            | 1.6               | 12.2          | 20.7                  | 53.7                     | 44.4                     |
| N.60.60         | 293.6            | 1.6               | 13.5          | 20.5                  | 67.3                     | 58.0                     |
| N.60.61         | 279.6            | 1.5               | 11.7          | 20.8                  | 58.5                     | 47                       |
| N.60.62         | 292.3            | 1.7               | 11.1          | 19.6                  | 52.1                     | 44                       |
| N.60.63         | 268              | 1.7               | 10.6          | 18.7                  | 49.1                     | 37.5                     |
| N.70.65         | 295              | 1.5               | 10.5          | 21.2                  | 33.4                     | 25.3                     |
| N.70.66         | 286.7            | 1.9               | 8.0           | 19.5                  | 60.4                     | 47.2                     |
| N.70.67         | 325              | 1.6               | 13.6          | 18.6                  | 53.2                     | 43.7                     |
| N.70.68         | 322.5            | 1.7               | 14             | 21.0                  | 61.6                     | 49.4                     |
N.70.69  288.8  1.7  11.6  17.3  41.2  34.2
N.70.72  291.3  1.6  10.2  20.5  55.5  46.3
N.70.73  319.6  1.6  11.8  19.9  63.4  52.9
N.70.74  292.7  1.5  10.6  18.3  46.9  39.4
N.70.75  289.3  1.5  10.7  16.0  36.1  30.1
N.70.78  329.6  1.7  14.5  20.7  49.2  40.6
N.70.79  311.3  1.5  13.5  22.0  57.8  42.2
N.70.81  330  1.8  11.7  21.5  56  45
N.70.82  331.4  1.8  12.4  18.5  63.4  53.1
N.70.83  286.5  1.6  10.7  19.5  65.8  53
N.70.84  298  1.5  11.6  21.1  44.8  28.6
N.70.85  315.5  1.7  12.1  21.2  43.8  32.2
N.70.86  320.4  1.5  12.1  20.1  64.7  53.1
N.70.88  291.3  1.5  9.2  21.8  66.8  53.1
N.70.90  310.7  1.8  11.3  21.2  73.3  64.4
N.70.91  302.2  1.6  10.6  20.7  34.9  25.8
N.70.92  316.7  1.4  14  20  47.2  42.0
N.70.94  305.6  1.4  12  20.8  61.6  50.7
N.70.95  305.7  1.5  12.6  20.3  60.5  47.3
N.70.96  314.5  1.6  13  18.1  31  23.4
N.70.97  272.6  1.5  13.3  20.2  69.3  59
N.70.98  300.3  1.5  11.1  21  56.1  49.3
N.70.99  334  1.4  11.4  21.1  56  43.5
N.70.100  313  1.4  14.2  20  67.3  52.8
Numbu  321  1.7  12.3  21.8  49.3  26.4

The Brix value in the observed M₃ mutant strain population showed considerable variation between 5-17%, while in the parent it was about 12%. Five putative mutants produce Brix value ≥ 15% ie N40.1.4; N40.10.3; N40.20.1; N40.20.2 (from 40 Gy radiation) and N50.39.12 (from 50 Gy radiation) (Table 1). Brix (sugar content) is total dissolved solids containing sucrose, fructose, and glucose. Selection of 11 genotypes of sweet sorghum performed by Pabendon et al.[1] produced Brix value about 9.67-12.6%, (10.02%), while in Numbu variety as control of about 9.03%. Brix value of sugar in sorghum varies greatly depending on variety, environment soil, water, climate, pests, and diseases), nutrient input and agronomic treatments [1].

Observations on plant height indicate the presence of strains that grow higher than the parents. Studied on the yield test of 10 putative Pahat-1 to 10 sweet sorghum mutant also produces strains that grow higher than their parents, higher plants will affect biomass [11]; [9].

The diameter of the stem becomes one of the selection criteria for obtaining a plant with a stronger morphological. Selected mutants with rod size range from 2-2.5 cm. The difference in stem diameter in the sorghum genotype can occur due to the genetic factors that predominate the plant's growth. Pabendon et al. 2012a [1] states that there is a correlation between plant height and stem diameter on the weight of stem biomass. Similarly, harvest time greatly affects the total biomass product and fermented carbohydrate content of sweet sorghum, thus the timeliness of the harvest is also very important to note.

3.2. Evaluation of Agronomic Characters and yield of 40 lines of M₃ mutants.

Observation of yield potential resulted in higher mutant strains compared to their parents, the same results occurred in M₃ strains (Table 1). The diameter of the middle stem ranges from 1.5-2 cm while the parent is around 1.8 cm, generally does not show any differences in the mutant lines tested (Table 2). Brix value 10%, compared to the M3 the putative mutant M₃ showed that some strains remained stable but some were decreasing, stable mutant lines produced above 14% Brix value can be seen in Table 3.
The important characters for the selection of sweet sorghum genotypes in ethanol production are the weight of biomass, the percent of Brix sugar, the volume of juice and the total high dissolved sugar. The standard deviation in panicle length ranged from 0.9 to 1.6 did not indicate a wide variation, the weight of wet and dry panicles showed a high standard deviation ranging from 10-30. In Table 2, we can see mutant lines that produce higher wet and dry panicle weight than parents.

Selection based on plant height, stem diameter, and content of Brix value in M₄ mutant lines resulted in 13 strains which were higher than parents, plant height ranged from 260-300 cm, stem diameter 2.7-3.0 cm, Brix value 13.7-17%, and the mutant strains were derived from irradiation with 40 Gray gamma rays (Table 3). The selection of the M₂ and M₃ mutant generation produced higher strains, plant height, stem diameter, Brix value content and, panicle production. The progeny (M₃) is expected to have stable agronomic characters and yield.

### Table 2. Agronomic characters and yield components of M₄ mutant lines

| Number of lines | Average/standard deviation |
|-----------------|----------------------------|
| N40-1-4-1       |                            |
| N40-1-4-2       |                            |
| N40-1-4-3       |                            |
| N40-1-4-4       |                            |
| N40-1-4-5       |                            |
| N40-1-4-6       |                            |
| N40-1-4-7       |                            |
| N40-1-4-8       |                            |
| N40-10-3-1      |                            |
| N40-10-3-2      |                            |
| N40-10-3-3      |                            |
| N40-10-3-4      |                            |
| N40-10-3-5      |                            |
| N40-10-3-6      |                            |
| N40-10-3-7      |                            |
| N40-10-3-8      |                            |
| N40-20-1-1      |                            |
| N40-20-1-2      |                            |
| N40-20-1-3      |                            |
| N40-20-1-4      |                            |
| N40-20-1-5      |                            |
| N40-20-1-6      |                            |
| N40-20-1-7      |                            |
| N40-20-1-8      |                            |
| N40-20-2-1      |                            |
| N40-20-2-2      |                            |
| N40-20-2-3      |                            |
| N40-20-2-4      |                            |
| N40-20-2-5      |                            |
| N40-20-2-6      |                            |
| N50-39-1-1      |                            |
| N50-39-1-2      |                            |
| N50-39-1-3      |                            |
| N50-39-1-4      |                            |
| N50-39-1-5      |                            |
| N50-39-1-6      |                            |
| N50-39-1-7      |                            |
| N50-39-1-8      |                            |
| Numbu           |                            |
4. Conclusion
Selection in the mutant population lines of $M_3$ and $M_4$ generation mutants gave positive results, that is an improvement in the characteristics of plant height, stem diameter and sugar Brix and panicle production. The results obtained indicate that the radiation treatment given produces mutants with the expected superior character.

Selection of mutant lines of sorghum $M_3$ and $M_4$ yielded 13 of $M_5$ mutant lines with better characters than Numbu ie at plant height (2.6-3.2m), stem diameter (1.6-2.0 cm), seed production (42-67 g) and Brix value (13.7-17%). Based on plant height, stem diameter and sugar Brix produces about 14 of $M_5$ lines that are ready for yield test and yield stability test.

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