Adaptation test and selection of numbu mutant sweet sorghum lines for character improvement of numbu variety

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Abstract. Sweet sorghum is a potential crop of agro-industry for food, feed and bioenergy. The availability of sweet sorghum varieties in Indonesia is still limited. One of the varieties that have a great demand is Numbu. Numbu has a large seed size, attractive color and contains juice in the stem, however it is still has weaken on small stems and low and unstable brix juice content. The efforts to assemble new superior varieties can be achieved through mutation breeding activities. Mutant lines produced from irradiation of Numbu varieties are expected to experience better character improvements than Numbu varieties. This study aims to find out the character improvements that occur in Numbu mutant lines which are tested in several different locations and to select potential Numbu mutant lines to be released as new superior sweet sorghum varieties. The research was conducted in five environments using 10 Numbu mutant lines and arranged into randomize block design with 3 replications. The results showed that the environment, genetic and interaction of genetic x environment had a significant effect for all observed, while the interaction of genetic x environments value for all observed characters have high variation depend on environment and environment gave the biggest contribution to variation, however generally mutant lines were have a better characters than Numbu variety. There is no important characters has stability performance in various environment, but they have a good performance on specific environment. Four mutant lines those have the potential to be released as new superior varieties were MNB3, MNB5, MNB8 and MNB10.

1. Introduction

Sweet sorghum (Sorghum bicolor (L.) Moench) is a cereal that widely planting and used as food, feed and bioindustry materials in many countries [1];[2];[3]. Sorghum in Indonesia mostly use as fodder and less people of Indonesia use it as staple food, because mostly Indonesian people eat rice, corn and other tuber crops [4]; [5]. Lack of public education about nutrition contents and functions of sorghum rice causes the market demand for sorghum rice to be less; therefore Indonesian farmers are still reluctant to plant sorghum. Not only the market and price uncertainty of sorghum rice, but also the biggest problem faced by Indonesian farmers is still limited sorghum variety circulating in Indonesia [6], so that farmers know only a few types of sorghum varieties. One of the most popular variety and often planted by
farmers in Indonesia is Numbu variety. Numbu is much favored by farmers because of its large spike, bigger seed size and sweet stems, but the Numbu variety still has many drawbacks, such as small stem diameter that easily fall when exposed to strong winds, unstable of brix content, juice volume and seed production. Improvement of Numbu character is very important to ameliorate the quality and quantity of sorghum in Indonesia.

The Improvement of plant character can be done by mutation method [7]. Mutation is one of the methods used to slightly improve the character of plants. Mutation breeding has the advantage of improving a defect in an otherwise elite cultivar, without losing its agronomic and quality characteristics [8]. The most common mutation is by gamma radiation. Gamma ray radiation can not only be done with a large number of samples, it is also very easy to do, very cheap and genetic changes can be adjusted depending on the dose and duration of radiation [9]. Numbu mutant lines that have been selected and have improved on the characters of stem diameter, juice volume, brix content and seed production are then expected to also have character stability which if planted in various environments will remain the same as those obtained in the preliminary and advanced tests as prerequisite for releasing varieties.

The process of releasing new genotypes for all crops needs a multi-location testing is a prerequisite including in Indonesia because Indonesia has various agroecosystem. Multi-location testing was carried out to examine the interaction of lines x environment and to select stable lines in various environments or adapt to specific environments [10]. The importance of identifying the factors causing the G x E interaction in determining breeding objectives, ideal conditions for testing and determining regional varieties that have good adaptations [11]. The Numbu-mutant sweet sorghum lines are expected to have improvements in a few important characters which are stable in various environments, therefore adaptation tests were carried out. Based on mentioned above the purpose of this study was to see the improvement of characters (brix, diameter, volume and yield) in Numbu mutant lines tested in various environments compared to Numbu variety and to select potential mutant lines based on important characters to be released as new superior sorghum varieties.

2. Materials and Methods
Ten Numbu mutant sweet sorghum lines derived from irradiated Numbu variety using gamma irradiation combined with in vitro culture used in this experiment were MNB1, MNB2, MNB3, MNB4, MNB5, MNB6, MNB7, MNB8, MNB9, MNB10 lines and Numbu variety as control. This research was conducted in May 2017 to November 2018 in five environments, (1) Maros (South Sulawesi), (2) Malang (East Java), (3) West Nusa Tenggara, (4) Lampung (South Sumatra) and (5) Pekalongan (Central Java).

| Regency and Province               | Soil types   | Height (mdpl) | Climate type |
|-----------------------------------|--------------|---------------|--------------|
| Maros, South Sulawesi             | Alluvial, dry| 27            | D2           |
| Malang, East Java                 | Andosol, dry | 10            | E1           |
| Sandubaya, NTB                    | Regosol, dry | 14            | D3           |
| Tegineneng, Lampung               | Alfisol, dry | 50            | C3           |
| Pekalongan, Central Java          | Brown As Alatosal, dry | 300 | C2 |

The research in each environment was arranged using randomized block design with three replications. Plot size was 2.5 m x 5 m and the planting distance was 75 cm between rows and 25 cm between plants in the row. Fertilizer applications were done twice, the first at 7 - 10 Days after planting (DAP) and the second at 30 - 35 DAP. Composite fertilizer NPK (15:15:15) about 300 kg / ha and Urea about 100 kg/ha is given to plant at first fertilization and at the second fertilization used only 150 kg/ha Urea, but before applying fertilizer must be ensured the land clean from weeds. Observation and
harvesting is done when the seeds enter the physiological mature phase that is all seeds are white and already hard.

The parameter observed were plant height (cm), stem diameter (cm), spike diameter (mm), juice brix content (%) measured using refractometer, volume of juice (liter), production (t/ha) and weight of biomass (t/ha). The collected data tabulated and analyzed using Microsoft Excel, STAR and PBT Tools software. The combined variance analysis use to see the interaction of genotypes and locations was carried out using the following mathematical model:

\[ Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \]

Where: \( Y_{ij} \) = observations; \( \mu \) = general mean value; \( \alpha_i \) = influence of the \( i \)-th location; \( \beta_j \) = influence of \( j \)-th genotype; \( (\alpha\beta)_{ij} \) = interaction of \( i \)-th location with \( j \)-th genotype; \( \varepsilon_{ij} \) = error

If the combined anova show significance influence, then continue with further tests of the least significance difference (LSD 0.05). Stability analysis was analyzed using GGE Biplot [12]; [13] with formula:

\[ Y_{ij} = \lambda_1 \xi_{i1} \eta_{1j} + \lambda_2 \xi_{i2} \eta_{2j} + \varepsilon_{ij} + \tilde{Y}_{i} \]

Where: \( \lambda_{1}, \lambda_{2} \) = singular Principal Component Axis (PCA) 1 and PCA 2, \( \xi_{i1}, \xi_{i2} \) = PCA1 and PCA 2 value for genotype \( i \), \( \eta_{1j}, \eta_{2j} \) = PCA1 and PCA 2 value for environment \( j \)-th dan \( \varepsilon_{ij} \) = residual value.

Stability Analysis was done using Plant Breeding Tools (PBTools) 1.3 Version.

3. Results and Discussion

3.1. Analysis of variance

Analysis of variance for the data combined across environment and lines presented in Table 2. The analysis clearly showed highly significant (P<0.01) and significant (P<0.05) difference for genotypes, environment and interaction between genetic and environment effects for the traits considered in the experiment. The environment was highly significant for plant height, stem Diameter, spike diameter, brix content, seed production and significant for biomass weight. The lines was highly significant for plant height, stem diameter, brix content, Juice volume, seed production, biomass and significant for spike. The genotype and environment interaction was highly significant (P < 0.01) for all observed Characters. Highly significant and significant of probability value by the influence of the environment, lines and interaction between them across the observation parameters showed that the environment and lines used in this experiment was very diverse.

Finding the lines that can show stable performance in various environments on the desired important character is very important. Indonesia has a diverse agro-ecosystem, so varieties that are able to appear stable in all environments are the main goal. Genetic improvement of the Numbu variety through mutation activities is expected to produce varieties with stable characters, considering that the Numbu variety is less stable in growth in various environments. Stable appearance of important characters in various environments is very important in order to obtain potential lines that can be extracted at various locations for special purposes such as juice volume and brix content. Genotype x Environment interaction values indicate the inability of line to have identical performance under different environmental conditions. Phenotypic variations rely on environmental changes and where all lines do not respond equally to environmental changes [14]. The average results in different environments are indicators of different lines responses and the presence of genotype-environment interactions [15].

In the Table 1 showed the relative contribution of each source variation Lines (L), Environment (E) and interaction between environment and Lines (EL). It was observed that environment was the most important source of variation for plant height (44.87%), stem diameter (61.92%), spike diameter (87.70%), brix content (39.56%) and juice volume (74.27%) traits. Interaction gave a great contribution on yield (40.48%) and biomass weight (27.37%) characters, meanwhile Lines only gave a great variation on plant height (33.55%) character. Among those various traits, Environment contributed minimum of 19.60 for Yield, where contribution of interaction was high (40.48%) and similar trend was observed for Biomass weight (27.37) and brix content (33.77) as well.
Table 2. ANOVA and proportion of variation (G + L + GL) explained by genotype (G), location (L) and GL interaction of sorghum Numbu mutant lines characters.

| Parameter                                      | Mean square     | CV (%)  |
|------------------------------------------------|-----------------|---------|
| Plant height                                  | 13098.68**      | 2.35    |
| Proportion of E, L & EL (%)                   | 44.87           |         |
| Stem diameter                                 | 287.76**        | 5.29    |
| Proportion of E, L & EL (%)                   | 61.92           |         |
| Spike diameter                                | 8507.19**       | 8.03    |
| Proportion of E, L & EL (%)                   | 87.70           |         |
| Brix content                                  | 58.75**         | 5.48    |
| Proportion of E, L & EL (%)                   | 39.56           |         |
| Juice volume                                  | 92532.86**      | 9.4     |
| Proportion of E, L & EL (%)                   | 74.27           |         |
| Yield                                         | 16.2471**       | 11.24   |
| Proportion of E, L & EL (%)                   | 19.60           |         |
| Biomass weight                                | 541.947*        |         |
| Proportion of E, L & EL (%)                   | 21.99           |         |

** and * = Significant at 0.01 and 0.05 probability levels, respectively

In the results of this study, there was not a single parameter that was not significant for the effect of interaction, this indicates that all parameters are sensitive characters to change if they are treated under different conditions, so that we cannot find a completely stable lines. The high interaction contribution to the characters of stem diameter, brix content, yield and biomass indicates that these characters are strongly influenced by cooperation between genetic and environmental actions. In other characters that have low interaction contribution values such as panicle diameter, plant height and juice volume generally have a very high environmental influence except for plant height where the influence of the genotype contribution is also high. By conducting stability tests, we can get specific lines that thrive in a certain environment using GGE, so we can develop mutant lines that are specific to use in a specific environment as well [16]. The purpose of mutation activity is to focus on changing the character of Numbu as food, feed and bioindustry in the specific environment where the variety will be developed later, so that better genetic improvement in characters related to the function of sorghum as feed, food and bioindustry becomes a benchmark for the success of research conducted.

3.2. Performance of sorghum mutan lines

The mean values of the mutan lines for the Characters considered are depicted in Table 3. Based on the results, the average agronomic characters and production of ten-MNB mutant lines were tested in five test environments on the characters of stem diameter, plant height, panicle diameter, sugar brix, sap volume, seed production and biomass weight are showed significant differences compared to Numbu variety. MNB 2, 3, 5 and 10 have a larger stem diameter. MNB-3,4,5,6,8 and 10 were the lines with the plant height better than Numbu and the average of all lines. With regards to Spike Diameter, MNB-2,4,5,6,7, and 9 were comparatively larger than average spike diameter of all lines. Character of Brix content was higher on MNB 5,6,7,8 and 10 lines. Juice volume is character that is closely related to bioindustry for bioethanol production, so that the lines that produce high volumes of juice are very good to be developed. MNB-3,4,5,6,8,9,10 are the lines those have a higher volume juice compare to average lines and Numbu variety. The four high yielding lines, MNB-3,5,9, and 10, had higher yield weight, 7.44, 7.48, 7.45 and 7.35 t/ha, respectively; however Numbu variety had the lowest (5.49 t/ha) yield weight. Likewise, MNB-10(50.45 t/ha) followed by MNB-8 (48.57 t/ha), MNB-7 (47.51 t/ha), MNB-3 (46.57 t/ha) and MNB-2 (46.42 t/ha) were the highest biomass weight lines.
The conclusion of the explanation the mutant lines above showed that the MNB-3,5 and 10 lines are the lines with the best performance on the average observed characters. The MNB-3 mutant line had the characteristics of stem diameter (15.67 mm), panicle diameter (59.17 mm), brix content (15.18) and production (7.44 t/ha). The MNB-5 mutant line had the highest stem diameter (16.01 mm), panicle diameter (62.34 mm) and production (7.48 t/ha) compared to the other nine mutant lines and significantly higher than the parents. The MNB-8 line had the highest plant height (300.56 cm) and Sugar brix (16.32%) compared to the other nine mutant lines and was significantly higher than the parental Numbu variety, while the MNB-10 mutant line had sap volume character. (144.48 ml) and the highest biomass weight (50.45 t/ha) compared to the other nine mutant lines and significantly higher than the Numbu variety. This showed that there is a genetic change for the better, especially those that are directly related to the improvement of characters for important sweet sorghum characters such as stem diameter, juice volume brix content dan yield.

**Table 3.** Character means of Numbu mutant sweet sorghum lines.

| Lines   | SD (mm) | PH (cm) | SpD (mm) | BC (%) | JV (ml) | Y (t/ha) | BMassW (t/ha) |
|---------|---------|---------|---------|--------|---------|----------|---------------|
| MNB-1   | 13.99 a | 276.82 a| 58.26   | 14.50  | 122.80 a| 6.90 a   | 44.80 a       |
| MNB-2   | 15.47 a | 266.12 a| 61.70 a | 14.79  | 123.84 a| 6.69 a   | 46.42 a       |
| MNB-3   | 15.67 a | 288.06 a| 59.17   | 15.18 a| 128.48 a| 7.44 a   | 46.57 a       |
| MNB-4   | 15.08 a | 283.66 a| 59.69 a | 15.15 a| 136.40 a| 6.51 a   | 46.39 a       |
| MNB-5   | 16.01 a | 280.34 a| 62.34 a | 15.56 a| 132.48 a| 7.48 a   | 47.51 a       |
| MNB-6   | 14.01 a | 280.14 a| 59.70 a | 15.95 a| 125.21 a| 6.70 a   | 45.25 a       |
| MNB-7   | 14.92 a | 265.63 a| 57.73   | 15.31 a| 102.87 a| 6.58 a   | 42.01 a       |
| MNB-8   | 14.09 a | 300.56 a| 59.64 a | 16.32 a| 143.19 a| 6.00 a   | 48.57 a       |
| MNB-9   | 15.05 a | 268.88 a| 59.72 a | 14.92  | 132.21 a| 7.45 a   | 45.53 a       |
| MNB-10  | 15.24 a | 300.19 a| 58.76   | 15.83 a| 144.48 a| 7.35 a   | 50.45 a       |
| Numbu   | 12.09   | 244.99  | 55.78   | 14.30  | 84.96   | 5.49     | 37.98         |

**Average** | 14.69 | 277.76 | 59.32   | 15.26  | 125.17  | 6.78     | 45.59         |

**CV** | 5.6 | 2.3 | 8.6 | 5.5 | 9.4 | 11.2 | 9.79 |

**LSD** | 0.60 | 4.72 | 3.45 | 0.62 | 8.52 | 0.55 | 3.23 |

Annotations : SD = Stem Diameter, PH = Plant height, SpD = Spike Diameter, BC = Brix content, JV= Juice volume, Y= Yield, BMassW= Biomass weight,
The lines followed by letter (a) were significantly different from the Numbu variety.

The increase characters of stem diameter, brix content, sap volume and yield that occurred in the MNB mutant compared to the Numbu variety was different at certain locations. There are certain locations were good for the development of certain characters in the test genotypes. Each location has its own influence on the character of growth and looks striking (Fig 1).

The average stem diameter character of the Mutant lines has a very good performance in Malang and Maros locations. The average character brix content of the mutant lines showed the best performance at Pekalongan, Maros and Malang locations. The average juice volume character of the mutant lines showed the best performance at the Maros and Sandubaya locations, while for the average yield character showed the best performance at the Tegineneng, Sandubaya and Malang locations.

Overall, Maros and Malang locations gave very good growth performance on MNB mutant lines for important characters observed. To find out how the distribution of observation data for these characters is carried out boxplot analysis to determine the distribution of observation data in locations where the characters are markedly increased.
In general, all Numbu-mutant lines experienced an increase of growth characters in all environments test compared to the Numbu variety. In the environment with significant growth and showed in boxplot. Boxplots can help us understand the characteristics of the data distribution and compare the distribution of several groups of data simultaneously. In addition to seeing the degree of spread of the data (which can be seen from the height/length of the boxplot) it can also be used to assess the symmetry of the data distribution. Knowing the data distribution of a line will make it easier for us to know the stability of the line (Fig 2). The length of the box describes the level of spread or diversity of the observational data, while the median location and the length of the whisker describe the level of symmetry. Based on boxplot analysis at best locations for character improvement, it can be seen that the stem diameter characters in Malang location showed that the MNB-3, 4, and 5 lines spread with a higher normal range than the other mutant lines and the Numbu variety.

On the brix content character at the Pekalongan location, the MNB-8 mutant genotype showed the best brix content performance. In terms of juice volume characters, the two locations with the best performance were Maros and Sandubaya locations, where the lines showed the best growth in these two locations and MNB-3, 6 and 8 lines because they are constant to produce juice in almost same volume. For yield character at Tegineng location, there is only one line that performs the best yield, it was MNB-1. Many locations have a good effect to perform yield character, but Tegineng perform the best one because many lines showed a high yield compare to other locations. Non-parametric measure can be used to see the stability of plant by seeing the data distributions [17].

Figure 1. Character performance of stem diameter growth, brix content, juice volume and yield at adaptation test locations
3.3. Stability analysis and lines selection using GGE-biplot

The results of the GGE analysis showed the value of PC. Based on the results of Table 4, it can be seen that the value of the sum of the mean squares of PC1 and PC2 were significantly different for the characters of stem diameter, sap volume, brix sugar content and yield, respectively with accumulated values of 95.2%, 84.6%, 71.6% and 70.7% respectively. The IPCA (Interaction Principal Component Axis) value of the influence of the line on the GGE analysis indicated the stability of the line in a certain environment [18]. The GGE biplot model was used to select and identify potential genotypes with a good character performance and adaptive to the environment [19]; [20].

Figure 2. Box plot of distribution and symmetry of observation data on stem diameter, brix content, juice volume and yield at the best test location
Table 4. Principal component value of GGE biplot analysis for stem diameter, juice volume, brix content and yield characters of Numbu mutant sweet sorghum lines

| Characters | Percent | Accumulation | Df | Mean.Sq | F.value | Pr.F |
|------------|---------|--------------|----|---------|---------|------|
| Stem Diameter |         |              |    |         |         |      |
| PC1 | 84.7 | 84.7 | 13 | 38.46561 | 56.92  | **   |
| PC2 | 10.5 | 95.2 | 11 | 5.658087 | 8.37   | **   |
| Juice Volume |         |              |    |         |         |      |
| PC1 | 58.2 | 58.2 | 13 | 4976.061 | 35.93  | **   |
| PC2 | 26.4 | 84.6 | 11 | 2666.698 | 19.25  | **   |
| Brix Content |         |              |    |         |         |      |
| PC1 | 48.2 | 48.2 | 13 | 9.600376 | 13.72  | **   |
| PC2 | 23.4 | 71.6 | 11 | 5.511206 | 7.88   | **   |
| Yield |        |            |    |         |         |      |
| PC1 | 46.5 | 46.5 | 13 | 7.012879 | 12.08  | **   |
| PC2 | 24.2 | 70.7 | 11 | 4.306392 | 7.42   | **   |

The GGE model that is applicable to form the GGE biplot is GGE-2. The X-axis (AEA) in the biplot-genotype GGE graph showed the average character of each genotype. The Y axis is a line perpendicular to the AEA axis and through the point of origin of the biplot which provides an overview of the agronomic stability of the Numbu mutant sorghum lines [21]. Further, genotypes that have higher performance than the average of all genotypes are on the right of the Y axis, while those on the left of the Y axis have yields below the average of all genotypes. Stable genotypes are those that are close to the X axis and the farther away the genotype is from the X axis, the more unstable it is [22]. The ideal genotype is indicated by a point on the AEA axis in the positive position [23]. The stable genotype is the closest genotype to the ideal genotype point than other genotypes in the biplot [24]. Figure 3 visualized the performance of stem diameter, juice volume, brix content and yield mean and stability according to the GGE biplot. The midpoint of the circle on the Average Environment Axis (AEA) line represented the ideal genotype. In the mean stem diameter of the G2 and G3 lines were close to the G5 ideal line. The mean juice volume and brix content showed the same manner, namely, G8 was the ideal line and G10 was close to the ideal line. The mean yield, the G1, G3 and G10 lines were close to the G5 ideal line. The order of stability based on the GGE biplot based on the 4 important characters were G5, G8, G3 and G10.
Figure 3. GGE biplots of the combined analysis for stem diameter, juice volume, brix content and yield (Mean versus stability of the genotypes)

GGE biplot is a very potential tool to analyze MET data to interpret complex GEI interaction [25];[26] It can effectively detect the interaction pattern graphically besides identifying ‘what-won-where’ and delineation of mega-environments among the testing locations [27]. However, this potential tool only a few research has been employed to analyze the multi-location data of sorghum important trait trials.

The what-won-where biplots indicated existence of crossover GE and existence of mega-environments, particularly for yield. ‘What-won-where analysis’ biplot of brix content showed there is no environment was close to the origin of the biplot. Yield polygon revealed only E5 environment that close to the origin, and the others (E1,E2,E3 and E4) being away from the origin. Juice volume and stem diameter showed the same manner that most of the environments being away from the origin, except E4. Out of the four what-won-where biplots, it may be observed that yield biplot (Fig. 2b) is the most informative, as it could discriminate environments more effectively and the polygon (hexagon) is well distributed. The biplot was divided into six sectors, four accommodating all the locations. The locations were partitioned into four groups. Group I was represented by E5 with G6, G7 and G8 as the nominal winner; Group II represented E2 with G10 as the winning genotype; Group III solely represented E4 where G1 and G3 were the nominal winners. Group IV was represented by E2 and E3, with G5 and G9 as the winning genotype. A genotype showing stability for one Character may not necessarily be stable for other character as well. This may be explained by the fact that each trait is governed by different set of genes and influence of environment on the cumulative expression of different set of genes will vary considerably, which is observed in variation in stability of genotypes for important characters.
4. Conclusions

Based on the results of the research, it can be concluded that breeding mutation can help to improve character of stem diameter, plant height, juice volume and biomass of sorghum mutant lines in various environment and showed higher performance compared to Numbu variety. There is no important characters has stability performance in various environment, but they have a good performance on specific environment. MNB3, MNB5, MNB8 and MNB10 mutant lines are the best lines to be released as new superior variety to be developed for specific need.

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