Atom Indonesia Vol. 37 No. 3 (2011) 102 - 106

Stability Test For Sorghum Mutant Lines Derived From Induced Mutations with Gamma-Ray Irradiation

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ARTICLE INFO

Article history:
Received 20 November 2010
Received in Revised form 01 August 2011
Accepted 05 August 2011

Keywords:
Gamma-ray irradiation
Genotypic stability
Grain yield
Mutant lines
Sorghum

ABSTRACT

Sorghum breeding program had been conducted at the Center for the Application of Isotopes and Radiation Technology, BATAN. Plant genetic variability was increased through induced mutations using gamma-ray irradiation. Through selection process in successive generations, some promising mutant lines had been identified to have good agronomic characteristics with high grain yield. These breeding lines were tested in multi location trials and information of the genotypic stability was obtained to meet the requirements for officially varietal release by the Ministry of Agriculture. A total of 11 sorghum lines and varieties consisting of 8 mutant lines derived from induced mutations (B-100, B-95, B-92, B-83, B-76, B-75, B-69 and Zh-30) and 3 control varieties (Durra, UPCA-S1 and Mandau) were included in the experiment. All materials were grown in 10 agro-ecologically different locations namely Gunungkidul, Bantul, Citayam, Garut, Lampung, Bogor, Anyer, Karawaci, Cianjur and Subang. In each location, the local adaptability test was conducted by randomized block design with 3 replications. Data of grain yield was used for evaluating genotypic stability using AMMI approach. Results revealed that sorghum mutation breeding had generated 3 mutant lines (B-100, B-76 and Zh-30) exhibiting grain yield significantly higher than the control varieties. These mutant lines were genetically stable in all locations so that they would be recommended for official release as new sorghum varieties to the Ministry of Agriculture.

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is a versatile crop and has big potential to be developed in dryland farming areas of Indonesia. It can be used as food source, animal feed, and raw materials for industries including biofuel (bioethanol). Therefore, if cultivated properly, sorghum might be able to increase land productivity and ensure food and energy security of the country. Unfortunately, not much research has now been done in sorghum breeding so that available superior genotypes adapted to the regions are still very limited. Sorghum breeding is of importance in order to improve productivity and quality, especially in lines with making optimal use of marginal or unproductive land.

Although hybridization is used to combine useful genes for the breeding of sorghum, the use of a mutation technique with gamma irradiation has been proven to provide effective methods in increasing plant genetic variability [1,2]. When combined with other breeding methods, this technique might be much more effective and efficient to produce superior genotypes. At National Atomic Energy Agency of Indonesia (BATAN), sorghum mutation breeding program for the improvement of drought and soil acidity tolerance has been reported to generate some promising mutant lines. These promising lines were reported to produce high biomass and grain yield when grown under adverse conditions of land *i.e.* drought prone and acid soil areas [3,4].

As regulated by the Ministry of Agriculture, sorghum breeding lines need to be tested in multi location trials before disseminated to the farmer society as a new sorghum variety. Particularly for sorghum, field trial requirements are needed at least

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in 10 agro-ecologically different locations, grown in two different seasons (dry and rainy seasons). Through these trials, sorghum adaptability and yield stability performances in a certain location can be evaluated. This is of importance in order to predict sorghum yield with regards to the existence of the genetic and environmental interactions.

Genotypic stability test is an important procedure in a plant breeding program especially for self-pollinated crops such as sorghum. Various methods of stability test have been developed by some biometrical experts such as Russel, Wilkinson and Tukey [5]. However, their approaches still remain problems with the high interaction variances since the judgment is simply taken by considering the linear component of the interactions. The *Additive Main Effect and Multiplicative Interactions* (AMMI) method has been developed further to overcome the problems, namely through partitioning the main interaction effects into its components [6]. The AMMI analysis make the assessment of genotypic stability be more reliable in a breeding program. The objective of this research is to study the grain yield and genotypic stability of some sorghum genotypes, including the mutant lines, in various locations by AMMI approach.

EXPERIMENTAL METHOD

Sorghum breeding materials available at the Center for the Application of Isotopes and Radiation Technology, BATAN were used in the experiment. Total of 11 sorghum genotypes consisting of 8 mutant lines derived from induced mutations through gamma-rays irradiation and 3 control varieties were included in the experiment (Table 1). All materials were grown in 10 agro-ecologically different locations namely Gunungkidul (L1), Bantul (L2), Citayam (L3), Garut (L4), Lampung (L5), Bogor (L6), Anyer (L7), Karawaci (L8), Cianjur (L9) and Subang (L10). In each location, the experiment used randomized block design with 3 replications.

Table 1. List of breeding materials included in the experiment.

Code	Name	Origin
G1	B-100	Seed irradiation (200 Gy gamma rays)
G2	B-95	Seed irradiation (300 Gy gamma rays)
G3	B-92	Seed irradiation (200 Gy gamma rays)
G4	B-83	Seed irradiation (300 Gy gamma rays)
G5	B-76	Seed irradiation (200 Gy gamma rays)
G6	B-75	Seed irradiation (200 Gy gamma rays)
G7	B-69	Seed irradiation (200 Gy gamma rays)
G8	Zh-30	Seed irradiation (300 Gy gamma rays)
G9	Durra	Parental variety from ICRISAT
G10	UPCA-S1	National control variety
G11	Mandau	National control variety

Data of grain yield (t/ha) from the multi location trials was used for analyzing descriptive statistics, analysis of variance and analysis of genotypic stability using AMMI approach [7,8]. The linear model for this experiment was denoted as:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + (\alpha\beta)_{ij} + \varepsilon_{ijk} \tag{1}$$

for $i = 1, 2,..,a$; $j = 1, 2,..,b$; $k = 1, 2,\ldots,r$

The effect of interaction between genotype and location was partitioned into several Components of Main Interaction (CMI) as follows:

$$(\alpha\beta)_{ij} = \sum\nolimits_{n=1}^{m} \sqrt{\lambda_n \, \nu_{in}}\, s_{jn} + \delta_{ij} \tag{2}$$

$\sqrt{\lambda_n}$ is a singular value for the bilinear component n^{th}, ν_{in} is effect of genotype i^{th} in bilinear component n^{th}, S_{jn} is effect of location j^{th} in bilinear component n^{th}. δ_{ij} is error from the bilinear modeling and m is the number of CMI having significance level at 5 % of probability. So in complete equation, the linear model became:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{k(j)} + \sum_{n=1}^{m} \sqrt{\lambda_n \, \nu_{in}}\, s_{jn} + \delta_{ij} + \varepsilon_{ijk} \tag{3}$$

for $i = 1, 2,..,a$; $j = 1, 2,..,b$; $k = 1, 2,\ldots,r$

The matrix interaction Z_{ij} was then formulated as:

$$Z_{ij} = Y_{ij} + \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j \tag{4}$$

with sum of squares of the interactions (SS_{ij}) as follows:

$$\begin{aligned} SS_{ij} &= r \sum Z_{ij}{}^2 \\ &= r \sum (Y_{ij} + \hat{\mu} - \hat{\alpha}_i - \hat{\beta}_j)^2 \\ &= r \sum (\bar{y}_{ij.} + \bar{y}_{...} - \bar{y}_{i..} - \bar{y}_{.j.})^2 \end{aligned}$$

Biplots were used to draw the AMMI analysis results in an ellipse with radius of r_i

$$r_i = \pm \sqrt{\lambda_i} \sqrt{\frac{2(n-1)}{n(n-2)} F_{2,n-2(\alpha)}} \tag{5}$$

AMMI Index Stability (AIS) values was determined by the following formula [9]:

$$AIS = \sqrt{\sum_{i=1}^{m} \left[\frac{\lambda_i^{1/2}}{\sum_{i=0}^{m} \lambda_i^{1/2}} \left(CMI\ Score_i \right) \right]^2} \tag{6}$$

Where λ_i was eigen value of the *i* interaction component, CMI score was the score of main component interaction (genotype), and *m* was the number of CMI having significance level at 5 % of probability.

RESULTS AND DISCUSSION

The average of grain yield (t/ha) of sorghum lines and varieties in 10 locatios were presented in Table 2. The first analysis was to confirm the assumptions required for valid analysis of variance (ANOVA). The analysis included testing for homogeneity of variance, normality of data and independency of the data errors. The results of Bartlett's and Levene's test for homogeneity of variance were presented in Fig. 1. It was shown that the p-values found from the two tests were not significant (p>0.05), meaning that we accepted the H_0 hypothesis i.e. the variances were homogeneous.

Table 2. The average of grain yield (t/ha) of sorghum genotypes in 10 locations.

Line & Variety	Location									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
G1	4.27	4.97	4.31	3.34	4.20	4.36	7.53	4.74	7.52	8.33
G2	3.37	2.86	4.07	3.03	3.96	3.71	5.19	3.20	6.06	6.91
G3	3.72	2.81	3.64	2.31	3.67	3.39	6.81	3.14	5.75	4.60
G4	3.80	2.80	3.80	2.55	3.43	2.87	4.96	3.82	4.78	6.97
G5	3.98	4.58	4.07	2.92	4.02	3.87	8.25	4.27	6.44	7.78
G6	3.14	3.90	3.49	2.90	3.63	3.18	4.90	3.32	6.50	7.95
G7	2.94	3.08	3.42	2.42	3.31	3.61	5.25	3.45	4.14	5.59
G8	4.54	5.38	4.87	3.72	4.42	5.34	8.88	5.16	7.15	8.41
G9	3.71	3.12	3.46	2.79	3.71	2.84	5.96	3.68	6.06	7.13
G10	3.16	2.77	3.10	2.60	3.03	3.48	4.61	3.33	5.01	6.67
G11	3.76	3.24	3.40	2.74	3.48	4.03	5.24	3.99	5.26	7.21

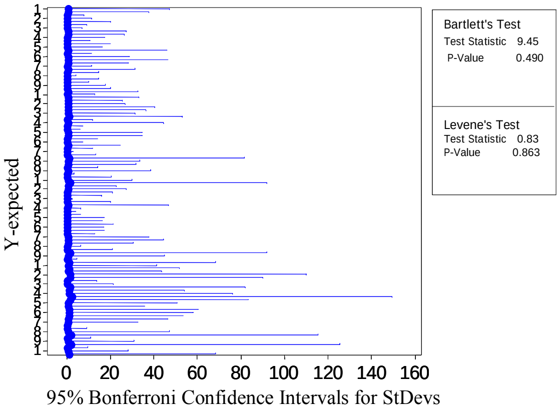

Fig. 1. Bartlett's and Levene's test for homogeneity of variance.

The results of Kolmogorov-Smirnov's test for normality of data were presented in Fig. 2. The Kolmogorov-Smirnov (KS) value was found to be 0.039 and it was not significant (p>0.05) so that the H_0 hypothesis was accepted (data errors distributed normally).

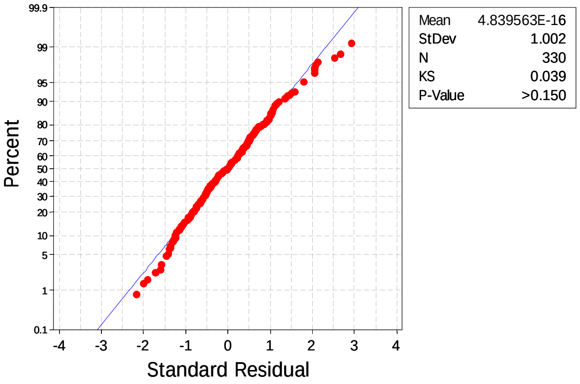

Fig. 2. Kolmogorov-Smirnov's test for normality of data.

The results of data errors independency test were presented in a plot of each data residual for their ordered observations as shown in Fig. 3. Since the plot did not follow any certain pattern, it could be concluded that assumption of data error independency was fulfilled.

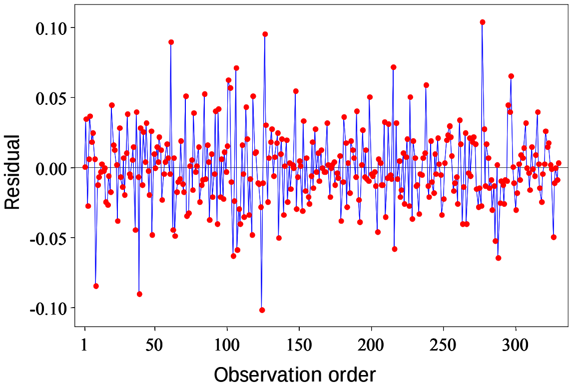

Fig. 3. The plot for data error independency test.

Based on the results above, all assumptions required for valid ANOVA were fulfilled so that the analysis of variance could be done accordingly. The grand ANOVA results were presented in Table 3. It was shown that all the main effects (Genotype and Location) and interaction (Gen x Loc) were significant at 5% level (p<0.05). In order to evaluate grain yield stability over locations, the $SS_{Gen\ x\ Loc}$ were partitioned into its Components of Main Interaction (CMI) and the results were shown in Table 4. The CMI1, CMI2 and CMI3 components were those of having significance at 5% level probability and cumulatively they contributed of 88.61% to the total SS of interaction (Table 4). Therefore, AMMI Index Stability (AIS) were sufficiently calculated by considering these 3 CMI components.

Table 3. The grand analysis of variance (ANOVA) for sorghum grain yield (t/ha).

Source of Variation	DF	SS	MS	Calculated F-value	P-value
Genotype	10	135.01	13.51	34.98	0.00
Location	9	579.11	64.35	166.6	0.00
Replication (Loc)	20	15.96	0.80	2.06	0.00
Gen x Loc	90	83.41	0.93	2.40	0.00
Error	200	77.24	0.39		
Total	329	890.80			

DF = Degrees of Freedom; SS = Sum of Squares; MS = Mean Squares

Table 4. Partition of the $SS_{Gen\ x\ Loc}$ into its Components of Main Interaction (CMI).

CMI	SS	df	Calculated F	Percent (%)	Accumulation (%)
CMI1	37.94	18	5.46*	45.53	45.53
CMI2	24.89	16	4.03*	29.87	75.41
CMI3	11.00	14	2.04*	13.21	88.61
CMI4	5.31	12	1.15	6.38	94.99
CMI5	1.95	10	0.51	2.35	97.34
CMI6	1.45	8	0.47	1.74	99.08
CMI7	0.48	6	0.21	0.57	99.65
CMI8	0.18	4	0.12	0.22	99.87
CMI9	0.11	2	0.14	0.13	100
Gen x Loc	83.41	90	2.19*	100	

*significant at 5% level of probability.

The distribution of lines and varieties (G) to their adapted locations (L) with positive or negative interactions could be interpreted from the AMMI-1 biplot as shown in Fig. 4. For example, 3 lines i.e. G1 (B-100), G5 (B-76) and G8 (Zh-30) exhibited relatively higher grain yield than the others and tended to adapt well in location L7 (Anyer) and L9 (Cianjur) since the interactions were positive.

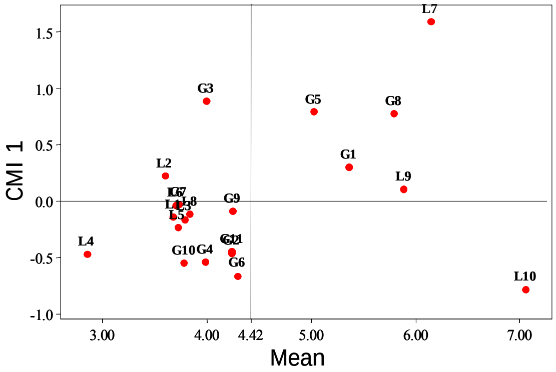

Fig. 4. AMMI-1 biplot of genotype interacted with location for the CMI1.

The lines G3 (B-92) was positively adapted at location L2 (Bantul) but the grain yield was low (below average). The control varieties (G9, G10 and G11) had low grain yield and interacted negatively with the location L1 (Gunungkidul), L4 (Garut) and L5 (Lampung).

Average grain yields of each line and variety over all locations were presented in Table 5. The stability of lines and varieties could be estimated from the AMMI-2 biplot which was made to configure the interaction structures between genotypes and locations (Fig. 5). This plot constituted agglutination of CMI1 and CMI2 in an ellipse of which both components explained of 75.41 % to the total variation (Table 4). A line and a variety was said being stable in all locations if its position was located inside the ellipse. Stability might also be interpreted from the AMMI Index Stability (AIS) values of each line and variety. The lower AIS value indicated that the corresponding lines and varieties were stable. Figure 6 shows the calculated AIS values for the corresponding lines and varieties. Therefore, it could be concluded from the two tables that all lines and varieties were actually stable except the G3 (B-92). As shown in Table 5, the stable developed lines G1 (B-100), G5 (B-76), and G8 (Zh-30) exhibited grain yield of 5.3573 t/ha, 5.0197 t/ha and 5.7868 t/ha, respectively, and they were significantly higher than the control varieties i.e. Durra (4.2457 t/ha), UPCA-S1 (3.7782 t/ha) and Mandau (4.2345 t/ha). Because of their stability, these promising lines were reliable enough recommended for official release as new sorghum varieties to the Ministry of Agriculture. Visual performance of the stable mutant line having the highest yield (Zh-30) was shown in Fig. 7.

Table 5. Average of grain yield (t/ha) for each genotype over all locations.

Code	Name	Grain Yield (t/ha)
G1	B-100	**5.3573^{d}**
G2	B-95	4.2362^{b}
G3	B-92	3.9855^{ab}
G4	B-83	3.9785^{ab}
G5	B-76	**5.0197^{c}**
G6	B-75	4.2907^{b}
G7	B-69	3.7230^{a}
G8	Zh-30	**5.7868^{e}**
G9	Durra	4.2457^{b}
G10	UPCA-S1	3.7782^{a}
G11	Mandau	4.2345^{b}

Note: numbers followed by the same letters are not significantly different.

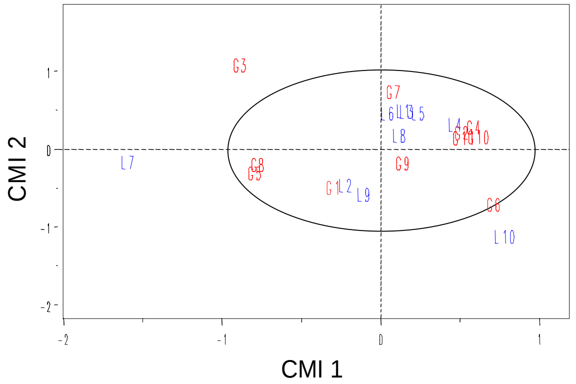

Fig. 5. AMMI-2 biplot configuring stabile genotypes inside ellipse.

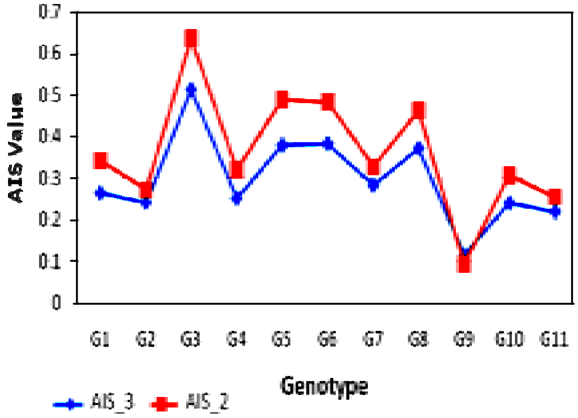

Fig. 6. AMMI-3 biplot of AIS values for the corresponding genotypes.

Fig. 7. Visual performance of the stable mutant genotype having the highest grain yield (Zh-30 line).

CONCLUSION

Sorghum grain yield data from the multi location trials met the requirements for valid analysis of variance (ANOVA). All main effects i.e. genotype, location and interaction (genotype x location) were significant. The interaction effects had been explored further for evaluating the genotypic stability over locations. It was concluded that all lines and varieties were stable except the G3 (B-92 line). Three stable lines i.e. G1 (B-100 line), G5 (B-76 line), and G8 (Zh-30 line), actually derived from induced mutations with gamma-ray irradiation, exhibited grain yield significantly higher than the control varieties. These promising stable mutant lines were reliable enough recommended for official release as new sorghum varieties to the Ministry of Agriculture.

ACKNOWLEDGEMENTS

Financial supports from PATIR-BATAN are greatly appreciated. Thank the Ministry of Research and Technology for including sorghum in the Incentive Research Program of 2007-2009 periods. Also, thank IAEA for supporting sorghum research program through the RCA-RAS/5/045 project.

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