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Yield Characteristics and Stability of Sugarcane Mutant in Three-Location Trials

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Abstract Bululawang (BL) has been cultivated annually under the increasing environmental pressure caused by global climate change. This condition often decreases the genetic ability and yields of agricultural products. Meanwhile, point 2.4 SDGs, specifically in sugarcane, can be achieved through a breeding program to improve and increase the genetic quality of several varieties of the plant in Indonesia. Mutation breeding to produce superior species has also been carried out by the Plant Breeding Laboratory, University of Jember, and this helped to increase the national sugarcane production. Therefore, this study aims to obtain new high-yielding varieties using the BL as the parent plant. The mutation breeding was carried out using the EMS mutagen, and this led to the production of three selected genotypes, namely M1.4, M2.4, and M3.4, which were further developed and tested for multilocation. The three selected locations for this study include Sukorambi-Jember, Arjasa-Jember, and Pakem-Bondowoso Districts. Based on the LSI test, M1.4, M2.4, and M3.4 showed the highest agronomic performance and yield in all locations compared to BL. M1.4 and M3.4 were identified to have high yield stability in the multi-location trials, hence, they were recommended as new stable superior genotypes. Therefore, M3.4 was declared as a site-specific adaptive species, which can be planted in the Arjasa and Sukorambi areas, based on the stability tests of Finlay and Wikinson, Eberhart, Russell, and AMMI.

Keywords Agronomic Performance, AMMI, Mutation Breeding, Sugarcane, Yield Stability

1. Introduction

The Climate Resilience Development Policy 2020–2045 and the Sustainable Development Goals (SDGs) revealed that the agriculture sector, specifically sugarcane plantations is vulnerable to climate change, which has been a significant problem for decades. Increased temperature causes variation in the water cycle, and this has an effect on evaporation, transpiration, and soil moisture processes. This condition can also facilitate heavy rains in a short time, which leads to a long dry season. Increased temperature promotes pests, diseases, and drought stress. This series of conditions have also been reported to increase the frequency of environmental stress for plants, specifically sugarcane.

In 2030, Indonesia has a target in the SDGs point 2.4, which ensures a sustainable food production system and implements resilient agricultural practices to increase harvest and protect the ecosystems. It also aims to improve soil and land quality as well as strengthen adaptive capacity to climate change, extreme weather, drought, floods, and other disasters. One of the best ways to achieve this goal is to develop new sugarcane genotypes that have broad adaptability. The development of new genotypes with conventional breeding is relatively difficult due to the difference in time needed for maturity in male and female

flowers. Previous studies revealed that mutation breeding is one of the best methods to overcome this problem.

Furthermore, this method is an alternative way to increase the genetic diversity in sugarcane plant. Mutagenesis in mutation breeding can be carried out using physical or chemical mutagens. The use of chemical mutagens in the process is a simple approach to creating plant genetic diversity, which is highly needed as a material for improving several potential agronomic traits [1]. Several studies revealed that mutation breeding using ethyl methane sulfonate (EMS) has been widely used to improve traits in several plant species [2].

Bululawang (BL) is one of the sugarcane varieties that is often cultivated by *BUMN,* a state-owned enterprise, as well as private, and smallholder farmers since 2004 [3]. The BL variety has a sugar potential yield of 7.51%, while that of sugarcane is 94.3 t/ha [4]. Sugarcane production in Indonesia is 70-75 t/ha or 2.6-2.8 million tons per year with a yield of 7-8%. Furthermore, the Indonesian Ministry of Industry reported that the national sugar demand reached 6 million tons per year, namely 2.7 million tons for consumption and 4.3 million tons for industrial needs. Based on these data, the country is still far from meeting the national needs of 3.2 million tons annually [5].

For 18 years, BL has been cultivated under abiotic stress, such as drought, floods, and high temperatures. These conditions were accepted as an impact of global climate change, which caused a decrease in the genetic ability and yields. Therefore, this study aims to carry out mutation breeding in the BL variety using EMS mutagen. Selected genotypes from mutations were evaluated in a greenhouse and cultivated in the field (*in vivo*). Agronomic parameters and yields are the main focus of this study.

2. Material and Methods

2.1. Callus Induction and Mutagenesis by EMS

Callus was induced with sterile shoot tip planting material from the BL variety sugarcane, which was cut to a thickness of 3-5 mm. Furthermore, induction was carried out using the MS medium with an additional hormone of 3 mg/l 2,4-D. The explants in the medium were placed in a dark room at 23 - 27°C. Mutagenesis using EMS was based on the method proposed by Koch [6]. The callus formed was then treated with EMS at a concentration of 16 mM for 5 and 10 hours. Its sub-culture was performed every 2 weeks for 18 weeks on a culture media. Callus that lived and grew until the end of the period was developed to form plantlets.

2.2. Evaluation of Mutant Sugarcane

Plantlets were grown in the greenhouse for up to 12 months, and each perfect plant was used as planting material in three locations, namely Sukorambi (Jember), Arjasa (Jember), and Pakem (Bondowoso) Districts. Furthermore, the mutant sugarcane was evaluated through agronomic characteristics, including stem diameter, plant height, number of tillers, Brix content, sugar content, and yield (t/ha).

2.3. Statistical Analysis

This study used a randomized complete block design (RCBD), with four replications at three experimental locations. Cultivation techniques were carried out based on sugarcane cultivation technical standards. Data normality and homogeneity were then analyzed using the Kolmogorov-Smirgov and Barlet tests. Furthermore, when the data were normal and homogeneous, analysis was continued using the variance and stability test combination. It was carried out to predict the interaction of genotypes and environment, based on the study by Gomez and Gomez [7]. When there was a significant effect among the genotypes, a one-way analysis, namely Least Significance Increase (LSI) was performed. The test was used to estimate the yield performance of each tested sample on the variety, following Petersen [8]. Stability test results were estimated based on Finlay-Wilkinson [9], Eberhart- Russel [10], and AMMI [11].

3. Results and Discussions

Table 1. Selected genotypes from mutagenesis with EMS

EMS concentration	Duration (hours)	Total	Genotypes code
16 mM	5	1	M1.4
16 mM	10	2	M2.4, M3.4

Based on the mutagenesis treatment using EMS, the best three genotypes, namely M1.4, M2.4, and M3.4 were found to survive up to 12 months. Furthermore, the selected genotypes grew perfectly and can be used as planting material in the evaluation of agronomic characters and yield components.

Table 2. The agronomic characteristics and t/ha yield production of the sugarcane mutant genotype

Agronomy traits	Genotype (G)		Location (E)		G x E		Replication	error	CV (%)
Stem diameter (cm)	0.62	**	0.05	*	0.02	ns	0.01	0.01	3.87
Plant height (m)	3155.02	**	7.18	ns	2.70	ns	15.20	53.73	2.71
Number of tillers	10.19	**	0.20	ns	0.04	ns	0.31	0.30	5.16
Brix (%)	3.58	**	0.24	ns	0.39	ns	2.16	0.23	0.69
Sugar yield (%)	4.10	**	0.02	ns	0.17	ns	0.39	0.13	3.36
Sugarcane yield (t/ha)	449.61	**	15.67	ns	62.86	*	15.26	21.50	4.45

Note: **) sign F. 1%; *) sign F. 5%; ns = non-significant; cv = coefficient of variation

Table 3. Sugarcane yield (t/ha) at each location and a combination of three locations

Genotype	Location						Mean (t/ha)	
	Arjasa		Sukorambi		Pakem			
M1.4	110.35	ab	111.09	a	108.34	a	109.93	ab
M2.4	100.08	a	110.08	a	99.66		103.27	a
M3.4	110.08	ab	103.16	a	109.12	a	107.45	a
BL	96.24		96.14		95.45		95.94	
BL + LSI (a)	100.93		102.17		102.52		101.53	
Mean + LSI (b)	108.88		111.15		110.21		109.73	
Mean	104.19		105.12		103.14		104.15	
LSI 5%	4.69		6.03		7.07		5.58	
CV (%)	3.47		4.43		5.29		4.45	

Note: Letters behind the numbers indicate that the tested genotype is superior to (a) BL and (b) the average mutant sugarcane genotype at 5% LSI test level.

The effect of genotypes and environment interaction was insignificant in all variables, except for the character of sugarcane yield. The association complexity was caused by variations in the various components of environmental factors [12]. However, the mean-squared value in the combined analysis of variance, namely the genotype, was higher than the mean square of its interaction with location for all variables. This indicated that the influence of genetic factors was more dominant than the environment.

The average sugarcane yields (t/ha) at each location and the combination of the three study areas are presented in Table 3. The 5% LSI test on the characteristics of sugarcane yields shows that the mutant genotypes of M1.4, M2.4, and M3.4 had superior performance at all locations, except M3.4, which was insignificantly different at Pakem, compared to BL variety. M1.4 had yield potentials of 110.35 t/ha (Arjasa) and 111.09 t/ha (Sukorambi), while values of 110.08 t/ha and 109.12 t/ha were obtained for M3.4 in Arjasa and Pakem, respectively. This indicates that the average harvest in all locations was 109.93 t/ha and 107.45 t/ha, which were significantly higher and superior to the BL variety. The M3.4 genotype mutant sugarcane had sugarcane yields (t/ha) of 100.08 t/ha at Arjasa and 110.08 t/ha at Sukorambi, which were also significantly higher and superior. However, at the standard location, it was only around 99.66 t/ha, which showed higher yields, but was non-superior to the BL variety. The average domestic sugarcane productivity was around 70 t/ha [4], while Riajaya & Kadarwati [13] showed that the production volume of the BL variety was 92.98 t/ha. This indicates that the production of mutant sugarcane is very high at >70 t/ha.

Table 4. LSI test results for agronomic traits of several mutant sugarcane genotypes at three locations

Genotype	Agronomy traits and yield component									
	Diameter(cm)		Height (cm)		Number of tiller		Brix (%)		Sugar yield (%)	
M1.4	3.422	a	285.556	a	11.708	a	17.996	a	11.138	a
M2.4	3.112	a	264.583	a	10.329	a	17.232		10.727	a
M3.4	3.198	a	281.111	a	11.108	a	17.780	a	11.073	a
BL	2.939		250.139		9.592		17.035		9.867	
BL + LSI (a)	3.042		258.966		10.256		17.419		10.300	
Mean	3.168		270.347		10.684		17.510		10.701	
LSI 5 %	0.103		8.827		0.664		0.384		0.433	

Note: Letters behind the numbers indicate that the genotype is superior to (a) BL and (b) the average mutant sugarcane genotype at the 5% LSI test level.

Table 5. The combined mean of sugarcane yield, regression coefficient, and regression deviation

Genotype	Mean of yield (t/ha)	$b_i = 1$		$b_i = 0$		S^2d_i	
M1.4	109.926	1.402	ns	1.402	ns	0.030	ns
M2.4	103.274	5.170	*	5.170	*	2.469	*
M3.4	107.454	-2.930	ns	-2.930	ns	1.609	ns
BL	95.943	0.358	ns	0.358	ns	0.017	ns
Mean	104.149	1.000		1.000			

Note: b_i = regression coefficient, S^2d_i = regression deviation, * = significantly different on the t-test of 0.05, ns = non-significantly different on the t-test of 0.05.

LSI was used to compare the mutant genotypes and BL as the parental variety. Furthermore, a significant difference between them showed a good performance of the genotypes, which can expand the selection opportunities of superior varieties [14], as shown in Table 4. M1.4 and M3.4 also had better agronomic characteristics of stem diameter, height, the number of tillers, brix, and yield compared to the BL variety. The M2.4 genotype showed better diameter, plant height, number of tillers, and yields than the parental variety. Meanwhile, M1.4 had the best value, followed by M3.4, and M2.4 in all parameters. Based on the characteristics, the brix content of M1.4 and M3.4 was significantly superior to the comparative varieties, but M2.4 showed no significant results and had lower content values.

A stable genotype was qualified with high adaptability in various environments when the regression coefficient value $(b_i) = 1$ and the mean yield value was higher than the total mean [9]. M1.4 and M3.4 had b_i values of 1.402 and -2.930, with a yield of 109.93 t/ha and 107.45 t/ha, respectively, as shown in Table 5. This indicates that they are stable genotypes with high adaptability level in all test locations.

Genotypes with $b_i = 1$ and average production yield were lower than the average total with stability and low adaptability levels in all test locations. The b_i value in the BL variety was 0.358, with an average production yield of 95.943 t/ha, as shown in Table 5. Based on this study, it was declared a stable genotype with low adaptability in all locations. Becker & Leon [15] revealed that when the regression coefficient has no different values, namely $b_i = 0$, the sample is classified in the static stability category. Therefore, M1.4, M3.4, and BL genotypes were classified in this category. M2.4 has a regression coefficient of 5.170 with an average production yield of 103.274 t/ha, as shown in Table 5. This shows that it has specific adaptability in an optimal environment, based on Finlay and Wilkinson's stability analysis.

The analysis of variance showed that the genotypes factor had a very significant effect on the sugarcane yield component in t/ha. The interaction between genotype and location significantly affected the yield in t/ha, as shown in Table 2. This indicates that the evaluation can be continued using AMMI analysis [16]. The AMMI model can be analyzed when there is an interaction between both factors [17]. The use of the model with the AMMI biplot was carried out to visualize genotypes that have stability in the test site or specific locations [18]. Genotypes are location specific when they are far from the main axis, but closer to the environmental line, while the stable variants are only close to the main axis [19]. These results were supported by Zhang [20] and Mustamu [21] that a stable genotype is close to the (0.0) level. Therefore, the samples that showed stability in three locations based on AMMI analysis were BL and M1.4, as shown in Figure 1a. A good genotype planted in a specific location e2, namely Sukorambi was

M2.4, while M3.4 was cultivated in e3 Pakem was M3.4. The interaction effect contribution explained by the AMMI 1 and AMMI 2 components was 99.3% and 0.7%, respectively, hence, the two components have a dominant share of explaining the association.

The biplot (What-won-where) visualizes the environmental sector division as well as the mega-environment, which helps to describe the appearance of the best genotype with the highest yield and shows the ideal location of a particular sample, as shown in Figure 1b [22]. The GGE biplot (What-won-where) showed that the BL and M2.4 genotypes had a yield in three locations. Meanwhile, M3.4 obtained the best sugarcane yield, when planted in Arjasa (e1) and Pakem (e3), and M1.4 had the best results at e2, namely Sukorambi.
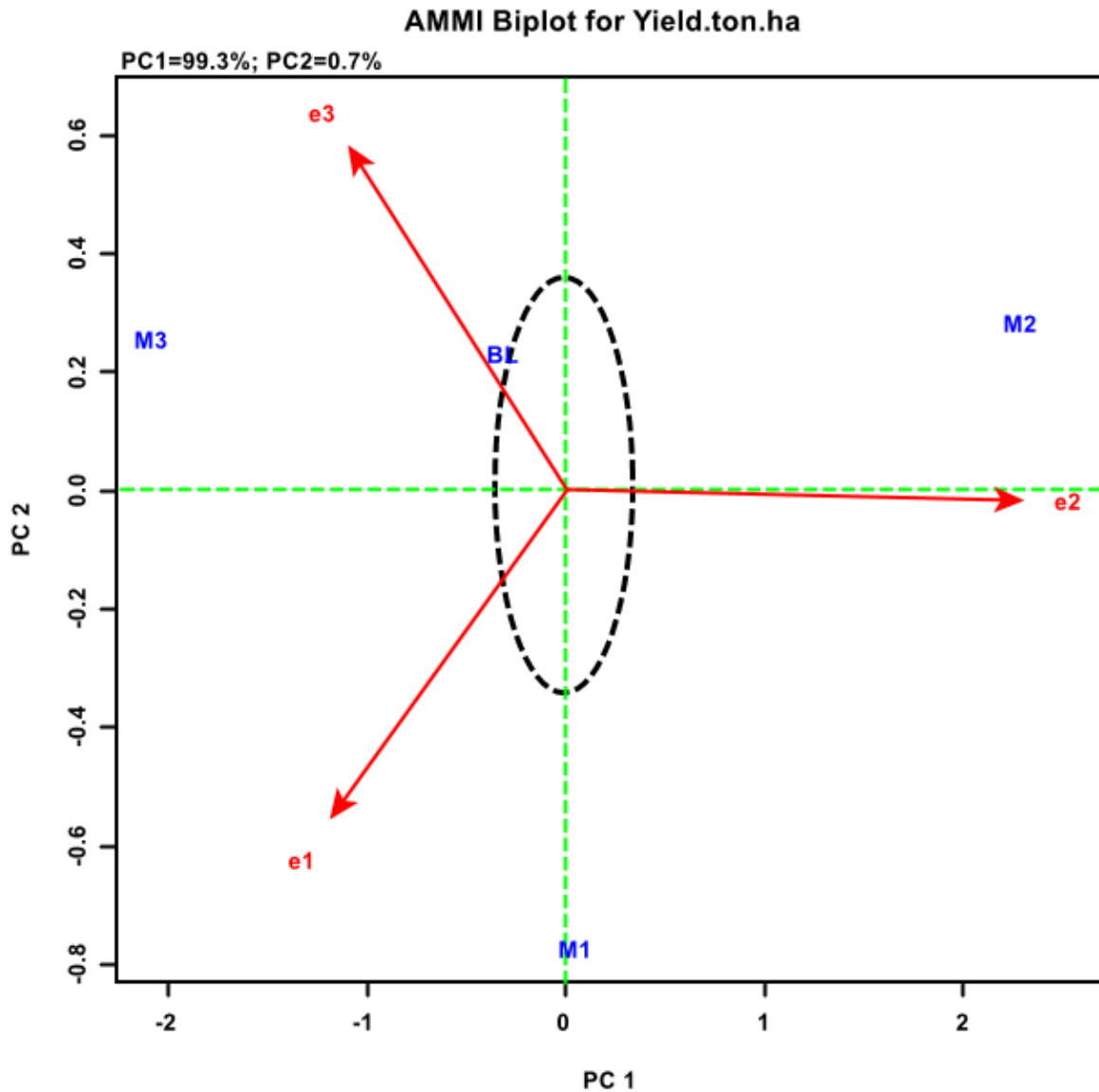
(a)
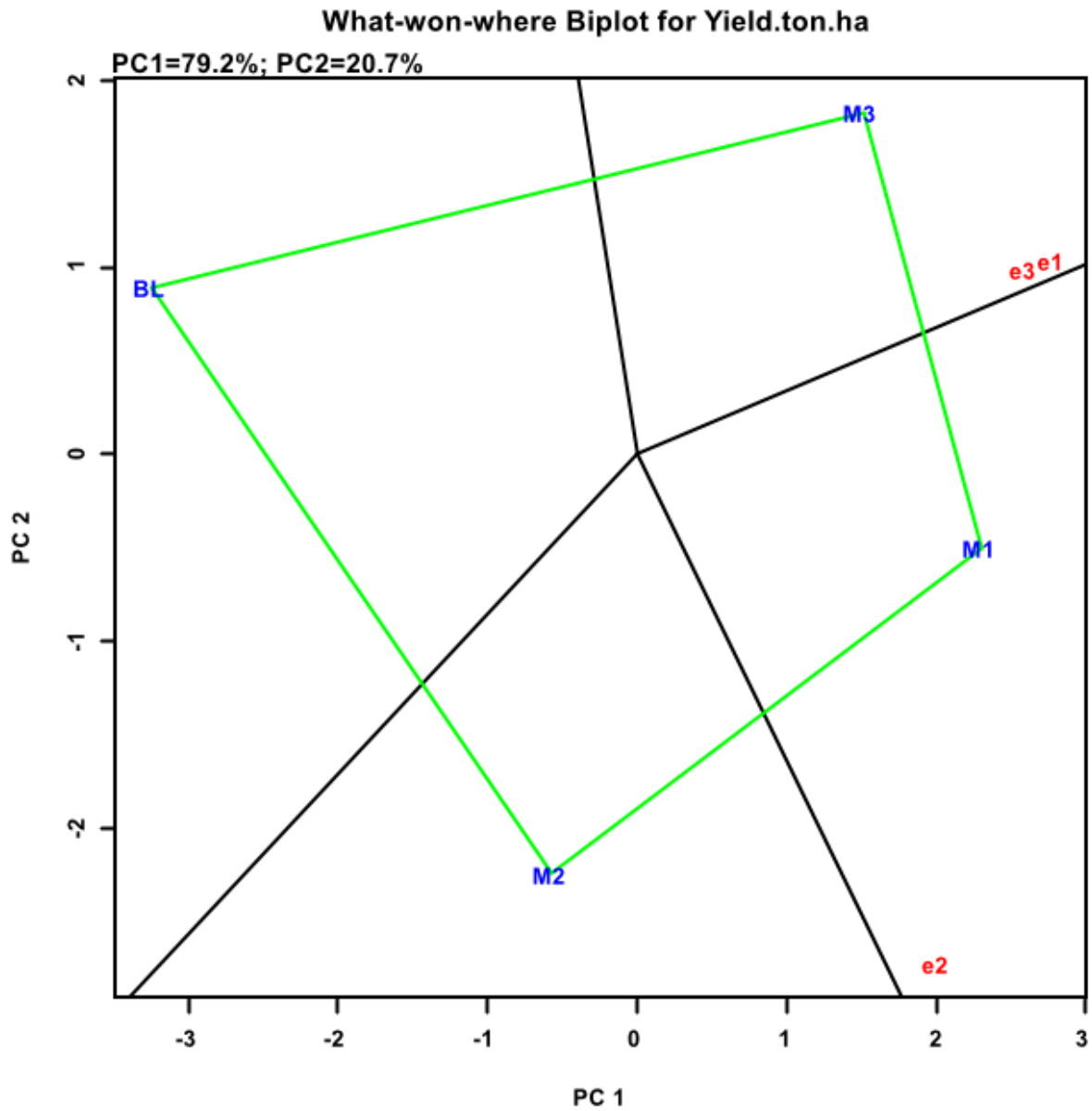
(b)

Figure 1. AMMI biplot PC1 x PC2 (a), Mega-environment biplot (b), yield components of four sugarcane genotypes at three locations; (e1) = Arjasa; (e2) = Sukorambi; (e3) = Bondowoso.

Eberhart & Russell [10] stated that a genotype is stable when the value of b_i is close to 1 and the S^2d_i in the regression deviation is close to 0. The regression coefficient (bi) of M1.4, M3.4, and BL was close to 1, and the regression deviation (S^2d_i) was close to 0, which indicates stability, as shown in Table 5. M2.4 had a regression coefficient above 1 with significant regression deviation, indicating that it is stable in a certain environment. Trustinah & Iswanto [23] also revealed that when the b_i is above 1 and S^2d_i is significantly different from 0, the sample is likely to have below-average stability with high sensitivity to environmental changes and the best results in an optimal environment.

Table 6. Three criteria for sugarcane yield (t/ha) stability

Genotype	Finlay & Wikinson	Eberhart & Russell	AMMI
BL	SS/LAAL	Stable	Stable
M1.4	SS/HAAL	Stable	Stable
M2.4	HAOE	-	Specific
M3.4	SS/HAAL	Stable	Specific

Note: SS= static-stable, HAAL= high adaptability in all locations, LAAL= low adaptability in all locations, HAOE= highly adaptable to an optimal environment.

Based on the analysis of variance, it can be concluded that the interaction of genotypes and environment can significantly influence the stability of sugarcane production [24]. Sugarcane genotypes with broad stability in the three locations can be used to maintain their genetic potential. This is expected to help them achieve the same production results in each test location despite the effect of the interactions [25]. BL and M1.4 were stable based on three yield stability analyses according to Finlay and Wikinson, Eberhart and Russell, and AMMI, as shown in Table 6. Furthermore, M3.4 was stable at all locations based on Finlay and Wikinson, as well as Eberhart and Russell, while M2.4 had location specific-stability according to AMMI analysis. Based on these data, BL, M1.4, and M3.4 had high stability against all test sites, and M3.4 wasstated as a specific-location genotype.

4. Conclusions

BL, M1.4, and M3.4 had high yield stability in all test sites, while M2.4 was declared to be location-specific based on the Finlay and Wikinson, Eberhart and Russell, and AMMI parametric tests. Based on the LSI 5% test of M1.4, M2.4, and M3.4 on stem diameter, height, the number of tillers, Brix, and yield, the values were better compared to BL. However, this study needs further improvement by analyzing the genetic diversity in sugarcane mutants based on the molecular biology approach.

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