



Mixed Model of Additive Main Effects and Multiplicative Interaction for Stability Analysis of Cassava

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Abstract: The research objective was to analyze Genotype \times Environment Interaction (GEI) using AMMI mixed model with Restricted Maximum Likelihood (REML) method both with and without coefficient of coancestry matrix (A matrix) assuming residual error variance across environments were homogeneous and heterogeneous. Multilocation trials were conducted at five districts of East Java Province, Indonesia, from November 2010 to August 2011. The results showed that no PCs values that significantly different from AMMI mixed model analysis, both without and with A matrix, assuming homogeneous error variance across environments. While the result of AMMI mixed model analysis, both with and without A matrix, assuming heterogeneous residual error variance across environments had the same interpretation. The most stable genotype that located closest to the origin of biplot was genotype G13 (CMM 02033-1). The yield potential of G13 was not high (close to average). Four genotypes namely G4 (Adira 4), G6 (CMM 03036-7), G7 (CMM 03036-5), and G15 (CMM 02048-6) were the most unstable genotypes. Environment S4 (Malang) had the smallest interactions effect, while environments with the greatest interaction effect were S3 (Probolinggo) and S1 (Kediri), because these environments had a long vector.

Keywords: Altitude, AMMI, cassava genotype, mixed model, stability analysis

1. INTRODUCTION

Cassava is able to adapt to various environmental conditions, but usually the adaptability of each variety is narrow and large, and it indicated the influence of genotype and environment interaction [1]. GEI (genotype \times environment interactions) cause limitations in selecting the superior genotypes, thus reducing benefits of the average analysis and conclusions become invalid [2].

Multilocation trials are necessary to:
(i) compare the appearance of genotype, i.e., genotype appearance in general (in many environments) and genotype appearance at specific environment;

(ii) estimate GEI component to measure the heritability and its impact on the selection;
(iii) selecting location of testing and determining the environment in a broader scope; (iv) identify genotypes with specific adaptation, as well as determining the purpose of breeding [3].

A wide statistical method has been developed to determine the genotype \times environment interaction. The most common method used is the combined analysis of variance. Then developed the technique of regression, univariate parameter stability (parametric and nonparametric stability), analysis of qualitative/crossover interaction, and

Multivariate analysis (cluster analysis, principal component analysis, factor analysis, Additive Main effects and Multiplicative Interaction (AMMI), and GGE biplot. The techniques of analysis developed in accordance with the development of multilocation trial data obtained. AMMI and GGE biplot get a lot of attention because of advantages in data interpretation of genotype x environment interactions compared to methods developed previously.

In fact, the data obtained from multilocation trials are often unbalanced, the variance across environments is not homogeneous, and there is the possibility of coefficient of coancestry among genotype used. AMMI is fixed model analyses with all the factors i.e environment, genotype, and their interaction are fixed. In its development, these factors can be random, so that mixed model is developed to analyze the genotype x environment interactions.

Model selection for GEI analysis is based on data obtained, i.e., by the presence or absence of heterogeneity of variance among environment, the data is balanced or unbalanced, the presence or absence of coefficient of coancestry between genotype, and so on. It is necessary to be conducted to get the best interpretation of the results based on data obtained from multilocation trials.

The research objective was analysis of GEI using AMMI mixed model with REML (Restricted Maximum Likelihood) method both with and without coefficient of coancestry matrix (A matrix) assuming residual error variance across environments were homogeneous and heterogeneous.

2. MATERIALS AND METHODS

2.1 Implementation of Research

The study was conducted at five locations: Kediri (80 m ASL), Ponorogo (800 m ASL), Probolinggo (40 m ASL), Malang (530 m ASL), and Mojokerto (25 m ASL), from November 2010 until August 2011. Experiments were conducted at each location using a randomized complete block design with three replications. Genetic materials of research

were 15 cassava genotypes, consist of 11 clones and four superior cassava cultivars as control involving Adira 4, UJ 5, Malang 4, and Malang 6.

Cassava was planted in a plot size of 5 m × 5 m with a spacing of 100 cm × 80 cm. Cassava cuttings about 20 cm long are planted with the vertically position of cuttings. Fertilization was given twice, at 1 month after planting with a dose of 100 kg ha⁻¹ Urea + 100 kg ha⁻¹ SP36 + 100 kg ha⁻¹ KCl, and at 3 months after planting with 100 kg ha⁻¹ Urea. Weeding was performed twice, at 1 month and 3 months after planting. The activities to improve the ridge were carried out before fertilization. Removal shoots with leaves two best buds performed at 2 months after planting. Harvest was conducted at 10 months. The character that observed was fresh tuber yield.

2.2 Statistical Methods

Linear mixed model equation used was $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{u} + \boldsymbol{\varepsilon}$ (Equation 1). Because of genotype, environment, and interactions were random, then linear mixed model equation became $\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_g\mathbf{u}_g + \mathbf{Z}_e\mathbf{u}_e + \mathbf{Z}_{ge}\mathbf{u}_{ge} + \boldsymbol{\varepsilon}$ (Equation 2) with \mathbf{y} = vector of parameters observed, $\boldsymbol{\beta}$ = a scalar of μ , \mathbf{u}_g = vector $n \times 1$ of random effect of genotype, \mathbf{u}_e = vector $g \times 1$ of random effects of location, \mathbf{u}_{ge} = vector $ge \times 1$ of random effects of genotype x environment interaction, \mathbf{X} = column vector whose elements are 1, \mathbf{Z}_g = incidence matrix ($n \times e$) which connects \mathbf{y} to \mathbf{u}_g , \mathbf{Z}_e = incidence matrix ($n \times e$) connecting \mathbf{y} to \mathbf{u}_e , \mathbf{Z}_{ge} = incidence matrix ($n \times ge$) which connects \mathbf{y} to \mathbf{u}_{ge} , $\boldsymbol{\varepsilon}$ = vector of random error. Random vectors \mathbf{u} and $\boldsymbol{\varepsilon}$ are assumed normal distribution and independent with zero mean [4, 5].

The combined analysis of variance was conducted using REML method based on two assumptions, namely homogeneous residual error variance across locations ($\sigma_1^2 = \sigma_2^2 = \sigma_3^2 = \sigma_4^2 = \sigma_5^2$) and heterogeneous residual error variance across locations ($\sigma_1^2 \neq \sigma_2^2 \neq \sigma_3^2 \neq \sigma_4^2 \neq \sigma_5^2$) [6, 7].

Data were analyzed using the SAS program i.e proc mixed for REML analysis without and with matrix A, proc IML for AMMI analysis (to obtain PC1 and PC2 score), proc inbred to obtain coefficient of coancestry among genotypes (A

matrix). U_{ge} value obtained from Equation 2 is used for the singular value decomposition and partition of AMMI analysis. Singular value decomposition of u_{ge} can be written $u_{ge} = \sum_{k=1}^t u_{ik} \lambda_k v_{jk}$, followed by the partition of singular value with formula $u_{ge} = \sum_{k=1}^t (u_{ik} \lambda_k^{1/2})(\lambda_k^{1/2} v_{jk})$, where $u_{ik} \lambda_k^{1/2}$ is PC score for genotype g_i in the k^{th} axis and $\lambda_k^{1/2} v_{jk}$ is PC score for environment e_j in the k^{th} axis.

3. RESULTS AND DISCUSSION

3.1 Tuber Yield

Average of tuber yield across environments were significantly different, with S1 (Kediri) had the highest yield mean of 54.84 t ha⁻¹ and S2 (Ponorogo) had the lowest tuber yield of 7.79 t ha⁻¹ (Table 1), so that S1 can be considered as the most productive environment and S2 was the least productive environment. Tuber yield in S2 (Ponorogo) was very low, it was may associated with altitude of experiment location i.e above 800 m ASL. According

to reference [8] states that cassava tuber yield was decreased in the highlands, which is caused by a decrease in the average of photosynthesis ability when cassava is cultivated in colder areas such as the highlands of the tropical and lowlands of sub-tropical. Cassava growth is slower in tropical highlands than in the lowlands; thus, it takes a longer period to obtain higher yields. Tropical lowlands have higher temperatures and strongly associated with plant growth and photosynthesis mean higher [9]. Growth and productivity of cassava require maximum temperature of 25 °C, high radiation and humidity, as well as adequate rainfall during the growing [10].

The mean value of 15 genotypes yield were tested in five environments was ranged from 23.95 t ha⁻¹ (genotype G11) up to 37.79 t ha⁻¹ (control varieties G3). Genotype G8 had yield mean of 37.52 t ha⁻¹, which was not significantly different from the control varieties G3, and higher than the other control varieties G1, G2, and G4. The yield mean in this study were higher than the results of [11] which tested 21 cassava genotypes in five environments

Table 1. Fresh tuber yield of cassava clones in each environment.

Code	Genotype	S1 ^a	S2	S3	S4	S5	Genotype mean
G1	UJ5	43.69	5.81	28.37	34.11	14.72	25.22 fg*
G2	Malang 6	62.83	9.64	37.12	37.81	15.43	32.58 bcd
G3	Malang 4	67.76	8.08	36.69	52.39	24.51	37.79 a
G4	Adira 4	56.79	8.23	43.58	29.29	18.97	31.51 cde
G5	CMM 03025-43	53.08	6.33	23.82	31.17	19.41	26.91 efg
G6	CMM 03036-7	67.26	9.83	26.27	33.70	21.57	31.52 cde
G7	CMM 03036-5	52.85	8.35	37.22	23.19	23.11	29.93 cdef
G8	CMM 03038-7	65.86	10.99	42.21	44.76	20.85	37.52 ab
G9	CMM 03094-12	46.26	3.79	25.95	39.06	11.02	24.40 g
G10	CMM 03094-4	58.58	9.18	29.74	52.00	23.18	34.55 abc
G11	CMM 03095-5	44.29	3.89	27.73	28.45	13.86	23.95 g
G12	CMM 02040-1	48.25	9.24	25.41	37.86	17.74	28.13 defg
G13	CMM 02033-1	53.52	8.13	30.70	40.51	13.11	29.49 def
G14	CMM 02035-3	60.22	4.72	26.34	27.35	13.80	24.16 g
G15	CMM 02048-6	41.40	10.66	23.90	31.17	22.88	26.69efg
Environment mean		58.54 p	7.79 t	31.00 r	37.08 q	18.28 s	29.59

^aS1 = Kediri; S2 = Ponorogo; S3 = Probolinggo; S4 = Malang; S5 = Mojokerto

*Mean values followed by different letters differ significantly ($P < 0.05$)

Table 2. The AMMI analysis result based on the REML method without and with *A* matrix assuming homogeneous error variance across environments.

	Eigen value (λ)		AMMI sum of square		Probability of H_0		Percentage		
	Without <i>A</i>	With <i>A</i>	Without <i>A</i>	With <i>A</i>	Without <i>A</i>	With <i>A</i>	Without <i>A</i>	With <i>A</i>	
λ_1	12.23	12.58	PC1	448.54 ^{ns}	474.65 ^{ns}	0.9201	0.8979	37.72	37.60
λ_2	12.09	12.26	PC2	438.21 ^{ns}	450.96 ^{ns}	0.8610	0.8454	36.85	35.72
λ_3	7.93	8.13	PC3	188.67	198.53	0.9906	0.9880	15.87	15.73
λ_4	5.84	6.46	PC4	102.46	125.03	0.9977	0.9943	8.62	9.90
λ_5	1.94	2.10	PC5	11.25	13.27	1.0000	1.0000	0.95	1.05
	Sum			189.13	262.44			100.00	100.00

H_0 : PCs were not affected ($PC_i = 0$)

ns = not significant in Wald test

PC = Principal Component

for two seasons with the yield ranges between 7.0 t ha⁻¹ to 17.9 t ha⁻¹ and lower than the reported by [12], who tested nine cassava genotypes in three locations with the range of yield between 26.4 t ha⁻¹ to 49.7 t ha⁻¹ with the average of 37.8 t ha⁻¹.

3.2. AMMI Mixed Model Technique without and with *A* Matrix Assuming Homogeneous Error Variance Across Environments

AMMI mixed model analysis without *A* matrix assuming homogeneous error variance across environments showed that PC1 and PC2 scores explained 37.72 % and 36.85 % of GE sum of square, respectively, and together its explained 74.57 % of the GE interaction variation (Table 2). But there are no PCs scores that significantly different, it was likely due to the small value of the AMMI sum of squares for each PC in this method compared with the Least Square method [13].

AMMI mixed model analysis without *A* matrix has not been reported on cassava, but it has been done in peanut [14] that assuming genotype as fixed effect and environment as random effect, in wheat [15] with different assumptions, namely genotype as a random effect and the environment as a fixed effect. The use of AMMI mixed models without *A* matrix provides better interpretation of genotype \times environment interaction [14].

Singular value decomposition on AMMI analysis of mixed models using *A* matrix result PC1 and PC2 scores with the cumulative proportion of 73.32 % (Table 2). Although the cumulative proportion of PC1 and PC2 were 73.32 %, but the PCs scores was not significantly different.

As AMMI, mixed model technique without *A* matrix, using the *A* matrix sum of squares AMMI also decreased, so that the PC values obtained no significantly different.

3.3 AMMI Mixed Model Technique without and with *A* Matrix Assuming Heterogeneous Error Variance Across Environments

AMMI mixed models analysis without *A* matrix assuming heterogeneous error variance across environment had proportion of PC1 and PC2 49.07 % and 24.47 % of the sum of squares of interaction, respectively. PC1 and PC2 scores explained 73.54 % of GE interaction. There were three PC values that significantly different on AMMI mixed model analysis without *A* matrix assuming heterogeneous error variance across environment (Table 3). This was in contrast to the results of the analysis using the assumption of homogeneous variance across environment where no PC scores were significantly different. If using *A* matrix assuming heterogeneous error variance across environment showed that singular value decomposition result PC1 and PC2 scores 47.79 % and 25.13 %, respectively, with a cumulative proportion of 72.92 %. In this method, three PC scores were also significantly different ($P < 0.01$) (Table 3).

Biplot AMMI1 both without and with *A* matrix assuming heterogeneous residual error variance across environments showed that genotype G11, G13, and G14 had the lowest of PC1 score among the other genotypes, so that those genotypes were most stable compared with other genotypes, but it has low yield potential (below the mean). Genotype G4 and G15 had the largest PC1 score, so

Table 3. The AMMI analysis result based on REML method without and with A matrix assuming heterogeneous error variance across environments.

	Eigen value (λ)			AMMI sum of square		Probability of H_0		Percentage	
	Without A	With A		Without A	With A	Without A	With A	Without A	With A
λ_1	12.24	12.55	PC1	449.58**	472.46**	0.00000	0.00000	49.07	47.79
λ_2	8.64	9.10	PC2	224.19**	248.45**	0.00035	0.00008	24.47	25.13
λ_3	7.52	7.87	PC3	169.58**	186.00**	0.00271	0.00100	18.51	18.82
λ_4	4.28	4.57	PC4	54.90	62.73	0.44519	0.32420	5.99	6.35
λ_5	2.45	2.51	PC5	17.94	18.93	0.93241	0.91913	1.96	1.92
Sum				916.19	988.57			100.0	100.0

H_0 , PCs were not affected ($PC_i = 0$)

**, Significantly different in Wald test 1 %

Table 4. The eigenvectors of genotypes and environments based on AMMI technique with the REML method without and with A matrix assuming heterogeneous residual error variance across environments.

Code	Genotype	Fresh tuber yield (t ha ⁻¹)	Without A		With A	
			PC1	PC2	PC1	PC2
G1	UJ5	25.22	-0.23	-0.67	-0.23	-0.62
G2	Malang 6	32.58	1.08	0.43	1.09	0.50
G3	Malang 4	37.79	0.67	0.81	0.72	0.65
G4	Adira 4	31.51	1.69	-1.00	1.73	-1.03
G5	CMM 03025-43	26.91	-0.82	0.46	-0.84	0.43
G6	CMM 03036-7	31.52	-0.54	1.57	-0.51	1.51
G7	CMM 03036-5	29.93	0.42	-1.05	0.51	-1.24
G8	CMM 03038-7	37.52	1.32	0.22	1.32	0.24
G9	CMM 03094-12	24.40	-0.20	-0.28	-0.23	-0.17
G10	CMM 03094-4	34.55	-0.49	0.51	-0.51	0.49
G11	CMM 03095-5	23.95	-0.07	-0.64	-0.12	-0.56
G12	CMM 02040-1	28.13	-1.04	-0.11	-1.06	-0.08
G13	CMM 02033-1	29.49	0.09	0.06	0.04	0.19
G14	CMM 02035-3	24.16	0.16	0.93	0.13	1.07
G15	CMM 02048-6	26.69	-1.86	-0.95	-1.83	-1.08
S1	Kediri	54.84	1.14	2.50	1.23	2.39
S2	Ponorogo	7.79	-1.27	-0.31	-1.14	-0.42
S3	Probolinggo	31.00	2.89	-1.30	3.00	-1.39
S4	Malang	37.08	-0.19	0.50	-0.19	0.48
S5	Mojokerto	18.28	-0.98	-0.61	-0.83	-1.02

categorized unstable. The environment that had the smallest interaction effect was S4 followed by S5, S1, S2, and S3. S4 was environment with smallest interaction effect with the second rank of potential yield (37.08 t ha⁻¹), while S3 was environment with the greatest interaction effect with the potential

yield 31.00 t ha⁻¹ (Table 4, Fig. 1, Fig. 2).

Biplot AMMI2 either without or with matrix A assuming heterogeneous error variance across environment also had the same interpretation. The Genotype having highest level of stability was

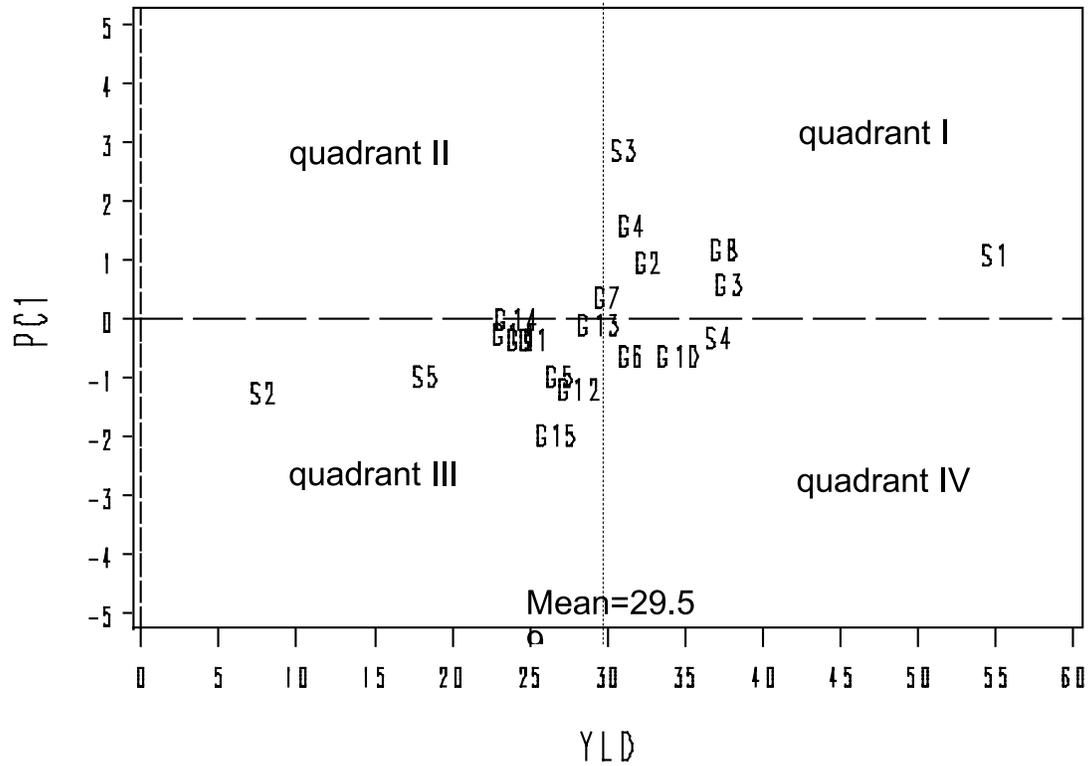


Fig. 1. AMMI1 biplot based on REML method without *A* matrix assuming heterogeneous error variance across environments.

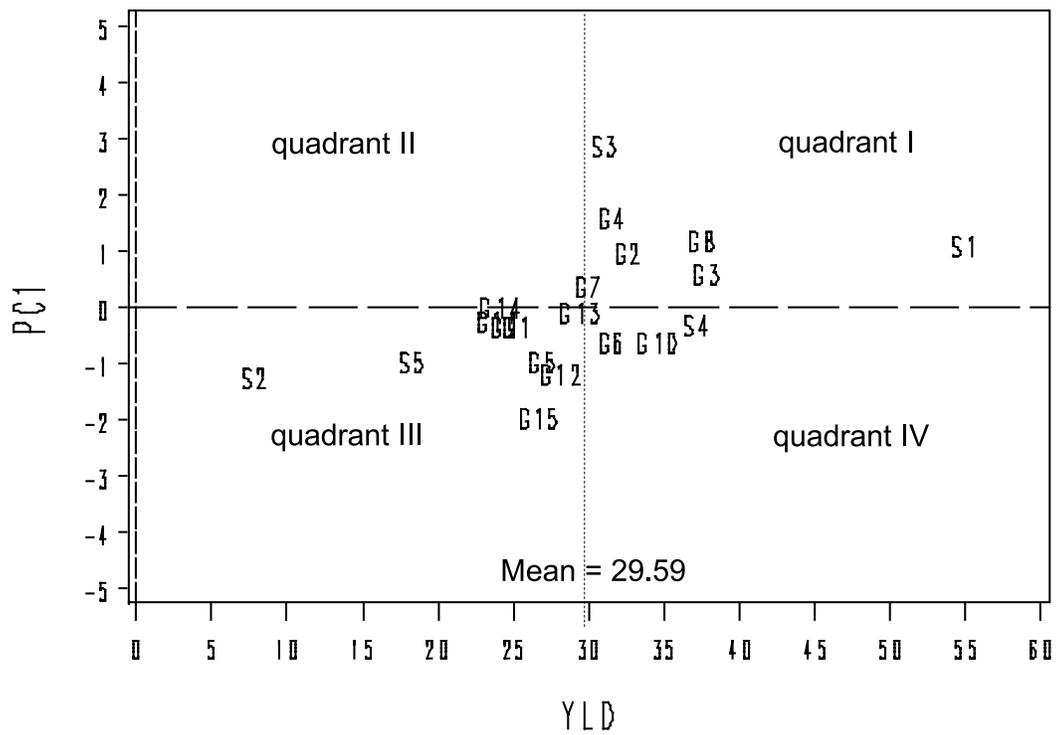


Fig. 2. AMMI1 biplot based on REML method with *A* matrix assuming heterogeneous error variance across environments.

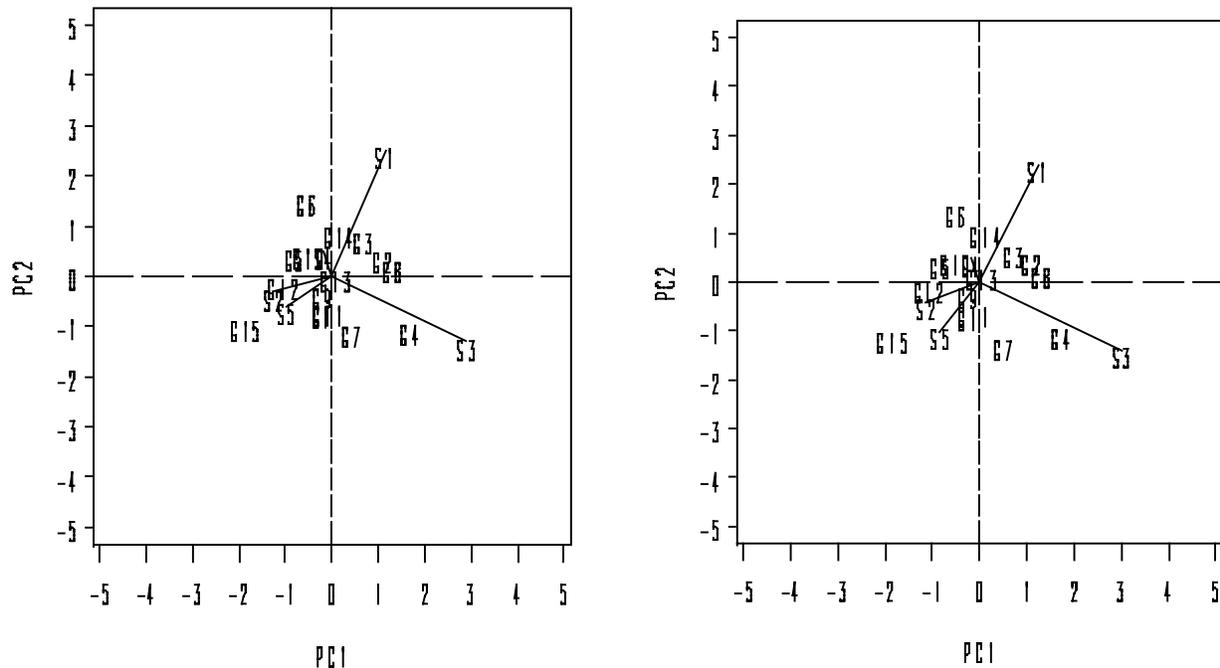


Fig. 3. AMMI2 biplot based on REML method without (left) and with A matrix (right) assuming heterogeneous error variance across environments.

genotype G13 because it was located closest to the biplot origin, but the potential results were not too high (slightly below the yield mean). Genotype G4, G6, G7, and G15 were the most unstable genotype because of its distance from the biplot origin than other genotypes. G10 was specific adapted genotypes in environments S4, G4 control varieties specific adapted to the S3, G12 was specific adapted to S2 (Fig. 3).

In both biplot AMMI2 (Fig. 3), it appears that the environment which had the smallest interaction effect compared with other environmental was S4, meaning that the yield potential of genotypes tested were not influenced by environmental factors in the environment S4. The environment that had highest interaction effect were S3 and S1, as it had long environmental vectors.

Scores PC1 and PC2 from AMMI analysis based on REML method without or with A matrix assuming homogeneous residual error variance across environments explained 74.57 % and 73.32 % of GE interactions variation, respectively, but no PCs scores were significant different. Using heterogeneous residual error variance assumptions, PC1 and PC2 explained 73.54 % of GE interactions variation (mixed model AMMI without A matrix)

and 72.92 % (mixed model AMMI with A matrix). The variation described declined compared with the Least Square method [13]. It can be visually seen on AMMI2 biplot obtained tends to closest to the biplot origin (0.0) compared with the Least Square method. This was in line with those reported by Sa'diyah et al. [16] that AMMI2 biplot based on mixed model AMMI was closest to the biplot origin. The two first PC scores on the mixed model AMMI obtained by reference [16] amounted to 44.49 %, less than the results of this study.

4. CONCLUSIONS

There were no PC scores that significantly different on AMMI analysis method based on REML method without and with A matrix assuming homogeneous error variance across environment. The results of AMMI analysis method based on REML method without and with A matrix assuming heterogeneous error variance across environment had the same interpretation. The most stable genotype was genotype G13 (CMM 02033-1) because it is located closest to the biplot origin, but the yield potential was not too high (close to the mean), whereas genotype G4 (Adira 4), G6 (CMM 03036-7), G7

(CMM 03036-5), and G15 (CMM 02048-6) were the most unstable genotype. Environmental S4 had smallest interaction effect, while environment with largest interaction effect were S3 (Probolinggo) and S1 (Kediri), because S3 and S1 had long environmental vectors.

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