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STEVIA (*STEVIA REBAUDIANA* B.) GENOTYPES ASSESSMENT FOR LEAF YIELD STABILITY THROUGH GENOTYPE BY ENVIRONMENT INTERACTIONS, AMMI, AND GGE BIPLOT ANALYSIS

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SUMMARY

Multilocation testing plays a vital role in the release of new high-yielding cultivars of stevia (*Stevia rebaudiana* B.) in Indonesia. In stevia, the leaf yield potential demonstrates an important characteristic for superior genotype selection. The study sought to identify the effects of genotype by environment interactions (GEI) on stevia yield and select the genotypes with stable yield resulting from radiation and hybridization through AMMI and GGE biplot analyses under three growing environments. The experiments took place in three locations, namely, Bandung, Sumedang, and Garut, West Java, Indonesia, consisting of a randomized complete block design (RCBD), with three replications. The combined analysis of variance (ANOVA) attained the genotype by environment interactions measurements. Calculating yield stability used the additive main effects and multiplicative interaction (AMMI), AMMI stability value (ASV), genotype stability index (GSI), and genotype plus genotype by environments (GGE) biplot measurements. The results revealed significant effects of environments (92.38%), followed by GEI (5.20%), and genotype effects (2.43%) of the total variation on stevia yield. The stevia genotypes viz., G11, G27, G2, and G5 gave a higher and more stable yield based on the AMMI-1 biplot. Based on the GGE biplot and the genotypes' sustainable performances, the stevia genotypes, i.e., G27, G2, G11, G20, and G26, gained selection as stable. The three selected stevia genotypes displayed the highest yields and proved stable in three environments viz., G2 (Tamangwangu EMS mutant number A), G11 (Bogor mutant with gamma ray radiation 5 number C), and G27 (a hybrid from Garut × Bogor-3). These promising genotypes exhibit the potential for further development into new superior stevia genotypes.

Keywords: Adaptability, GEI, multilocations, productivity, West Java-Indonesia

Key findings: Increased stevia leaf yields proved highly influenced by genotypes, environments, and their interactions, where environmental effects contributed 92.38% of the total variation. The use of various stability measures, including AMMI and GGE biplot, helped select three superior stevia genotypes as being stable and high yielding under various environments..

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INTRODUCTION

Stevia (*Stevia rebaudiana* B.) is a potential natural sweetener and is estimated to be 200–300 times sweeter than sucrose (Zhang and Bell, 2017). Steviol glycosides, viz., stevioside and rebaudioside-A, are found in stevia and can treat various diseases, such as, cancer, diabetes mellitus, and obesity (Momtazi-Borojeni *et al*., 2016). In addition, glycoside contents confirmed as very effective in the reduction of blood glucose levels (Ritu and Nandini, 2016).

Multilocation trials play an imperative role in screening crop genotypes for potential yield in plant breeding programs (Al-Taweel *et al*., 2019, 2021; Maulana *et al*., 2022). According to Abakemal *et al*. (2016), multilocation trials aim to select stable maize genotypes in various environments. Ajay *et al*. (2020) conducted multilocation trials to estimate the GEI effects and yield stability in peanuts. In another study, Ruswandi *et al.* (2022) also estimated GEI effects and genotypic stability in sweet corn by a multilocation test using cultivar stability measurements. Therefore, multilocation-genotypes testing is one of the crucial objectives for researchers in selecting superior genotypes.

Genotypes with high yield and stability in various environments serve as key parameters expected by researchers, farmers, and industrial users in barley (Vaezi *et al*., 2019) and orange-fleshed sweet potato (Maulana *et al*., 2020). However, yield stability can be measured through various stability measurements, including linear regression (bi and S_2di) (Eberhart and Russell, 1966), the Wricke's ecovalence (Wricke, 1962), Shukla stability value (Shukla, 1972), coefficient of variation (CVi) (Francis and Kannenberg, 1978), non-parametric measurements (Huehn, 1990; Thennarasu, 1995; Maulana *et al*., 2022), AMMI (Gauch, 2013), and GGE biplot (Ruswandi *et al*., 2021) analyses. The use of AMMI and GGE biplot analysis can combine and separate genotypes, environments, and their interactions (GEIs) in multi-environment trials. In addition, these two analyses have good visualization in determining the stability and adaptability of the tested genotypes (Yan *et al*., 2007; Gauch *et al*., 2008). Several researchers succeeded in selecting more stable and high-yielding genotypes by using various stability measurements, including Vaezi *et al*. (2019) in barley, Ruswandi *et al*. (2022) in sweet corn, and Karuniawan *et al*. (2021) in orange-fleshed sweet potato. According to Vaezi *et al*. (2017), using only one stability measure proved less informative and unhelpful in selecting more stable and high-yielding genotypes. Therefore, it is necessary to use various stability measurements in order to select the ideal, more stable, and high-yielding genotypes through multilocation testing.

Growing environment variations can cause differences in the yield potential of the tested genotypes. Some researchers suggested that environmental effects, genotypes, and their interaction effects can lead to the variation in yield under each environment in maize (Mafouasson *et al*., 2018; Ruswandi *et al*., 2022). In other studies, different soil conditions can also influence the genotype responses of yield in crop plants (Prayoga *et al*., 2021). Tolorunse *et al*. (2018) reported that significant environmental differences are the critical cause of the emergence of GEI in soybean and other crops. Therefore, the existence of GEI can make the plant selection process difficult.

Leaf yield stability is one of the essential genotypic parameters in stevia (*Stevia rebaudiana* B.). Hence, it is necessary to select the stevia genotypes in each environment to obtain area-specific genotypes and test for leaf yield stability to know the genotypes that can adapt to various environments. In previous studies, stevia leaf yield exhibited the highest heritability and genetic gain that could be passed on to the next generation (Amien *et al*., 2021). In another study, the sweetness level (Steviol glycoside) of stevia received positive influences from the genotypes, plant age, planting year, and also their interactions, and tended to increase when the plants grew older (Barbet-Massin *et al.*, 2015). The selection of new stevia genotypes based on leaf yield traits seemed to be a solution to the development of stevia crop in Indonesia. So far, information regarding the stability of stevia leaf yields is very few. With leaf yield being one of the crucial traits in plant cultivation, it serves as one of the reasons farmers choose a genotype. The recent research is very important as it will provide information regarding more stable and high-yielding stevia genotypes, as well as, the best locations for its wider cultivation. Therefore, the latest study aimed to identify the GEI and select the stevia genotypes by using several stability measures in three different environments in West Java, Indonesia. The outcome of this research hopes to fulfill the farmers' needs to cultivate more stable and high-yielding stevia genotypes in Indonesia.

MATERIALS AND METHODS

Plant material and experimental design

The plant material comprises 31 stevias (*S. rebaudiana* B.) genotypes (16 genotypes obtained through mutation, with five from ethyl methane sulfonate (EMS) and 11 from gamma rays with doses between 3.0–7.5 k Rad, and 12 genotypes attained through hybridization) and three check cultivars (Table 1). The checks used are local cultivars used as parents of mutants and cross-bred stevia. In stevia genotypes, three locations in West Java, Indonesia, served as the study areas for the leaf yield stability, i.e., Bandung (Cibodas Village, Ciwidey District), Sumedang (Jatinangor, Sumedang), and Garut (Mekarsari Village, Cikajang District) (Table 2). The three environments used are stevia development areas in West Java, Indonesia. The experiment at each location set up a randomized complete block design (RCBD) with three replications during 2019. The plot size used measured 2.5 × 5 m^2, with a spacing of 25 cm × 25 cm. Each plot consists of 200 plants for each genotype.

Table 1. Stevia genotypes used in the study at the three locations of West Java, Indonesia.

No.	Treatments	Code	Genotypes	Information
1	Mutation	G1	GR 3,5 B	Garut gama ray radiation 3,5 no B
2		G2	TEA	Tawangmangu EMS no A
3		G3	GR 7,5 A	Garut gama ray radiation 7,5 no A
4		G4	TR 3,5 B	Tawangmangu gama ray radiation 3,5 no B
5		G5	BEB	Bogor EMS no B
6		G6	GR 5 B	Garut gama ray radiation 5 no B
7		G7	BR 5 B	Bogor gama ray radiation 5 no B
8		G8	GEA	Garut EMS no A
9		G9	BED	Bogor EMS no D
10		G10	TR 3,5 C	Tawangmangu gama ray radiation 3,5 no C
11		G11	BR 5 C	Bogor gama ray radiation 5 no C
12		G12	GR 3,5 C	Garut gama ray radiation 3,5 no C
13		G13	GR 7,5 B	Garut gama ray radiation 7,5 no B
14		G14	TED	Tawangmangu EMS no D
15		G15	BR 5 D	Bogor gama ray radiation 5 no D
16		G16	BR 5 A	Bogor gama ray radiation 5 no A
17	Crosses	G17	STG 1	Tawangmangu x Garut 1
18		G18	STG 7	Tawangmangu x Garut 7
19		G19	STG 8	Tawangmangu x Garut 8
20		G20	STG 10	Tawangmangu x Garut 10
21		G21	SBG 3	Bogor x Garut 3
22		G22	SBG 4	Bogor x Garut4
23		G23	SBG 5	Bogor x Garut 5
24		G24	SBG 7	Bogor x Garut 7
25		G25	SBG 10	Bogor x Garut 10
26		G26	SGB 2	Garut x Bogor 2
27		G27	SGB 3	Garut x Bogor 3
28		G28	SBT 11	Bogor x Tawangmangu 11
29	Check	G29	BK	Bogor (Check)
30		G30	TK	Tawangmangu (check)
31		G31	GKA	Garut control (check) nomor A

Table 2. Meteorological data and soil properties of the study locations of West Java, Indonesia.

Locations	Coordinates	Alt.	Temp.	RF	Hum.	Soil parameters					
						Soil ordo	pH H_2O	C (%)	N (%)	C/N	P_2O_5 HCl 25%
Bandung	7°05'34.8'S 107°29'07.8"E	1100	22.00	369.80	69.00	Andosols	6.59	2.95	0.41	7	61.25
Sumedang	6°54'59.6"S 107°46'14.5"E	530	25.00	275.30	64.00	Inceptisols	5.34	2.04	0.23	9	35.55
Garut	7°21'01.8"S 107°48'01.5"E	1200	24.82	203.30	72.67	Andosols	5.01	3.16	0.15	21	70.46

Alt. = Altitude (m.a.s.l.), Temp. = Temperature (°C), RF = Rainfall (mm), Hum. = Humidity (%)

Data collection

Recording of the fresh leaf weight (kg) and leaf yield data (kg per plot) of the stevia genotypes took place at harvest time. Harvesting happened three times, namely, 60 days after planting, 90 days after planting, and 120 days after planting.

Data analysis

Genotype by environment interaction (GEI) analysis ensued for all the stevia genotypes. The statistical model for combined ANOVA used the following:

$$Y_{efgh} - \mu = G_e + R(G)_{e(f)} + E_g + GE_{eg} + \varepsilon_{efgh}$$

Where:
Y_{efgh} is the measurement on plot h in field trial e, block f containing genotype g, μ is the grand mean of all plots in all field trials, G_e is the effect of field trial i, $R(G)_{e(f)}$ is the effect of block (replication) j in field trial i, E_g is the effect of stevia genotype k, GE_{eg} is the genotype (i) with field trial (k) interactions, and eijkl is the plot error.

The estimation of the contribution to total variation (%) used the following formula:

$$\text{Contribution to total variation } (X_i) = \frac{X_i}{X_g + X_e + X_{gei}} \times 100\%$$

Where:
X_i is the ith item (sum of square [SS]) in the set; X_g is the SS value of genotypes (G); X_e is the SS value of environment (E); and X_{gei} is the SS value of genotype by environment interactions (GEI).

To classify genotypes in the same group, the Duncan Multiple Range Test (DMRT) was used following Harter (1960):

$$DMRT = R_{p;dfe;\alpha}\sqrt{\frac{MSE}{r}}$$

Where:
p is the rank distance of the two treatments; dfe is the degree of freedom for error; α is the significant level; MSE is the mean square error; and r is the repeatable.

The AMMI stability model estimated the stevia genotypes' yield by following Gauch (2013):

$$Y_{ef} = \mu + Ge + E_f + \sum_{k=1}^{n}(\lambda_g\ \alpha_{eg}\ \gamma_{fg}) + \rho_{ef}$$

Where:
Y_{ef} is the yield performance of the genotype eth in the environment fth, μ is the average of all yield performances from genotypes used, G_e is the genotype eth mean deviation, E_f is the environment fth mean deviation, λ_k is the square root of the eigenvalue of the PCA axis *g*, α_{eg} and γ_{fg} are the PC score for PCA axis *g* of the genotype ith and the environment fth, respectively, and ρ_{ef} is the residual.

The AMMI stability value (ASV) estimation used the following formula:

$$ASV = \sqrt{\frac{ss\ IPCA1}{ss\ IPCA\ 2}(IPCA\ 1\ score)^2 + (IPCA\ 2\ score)^2}$$

Where:
ss IPCA1 and ss IPCA2 were the weights given to the IPCA 1 and IPCA 2 scores by dividing the ss IPCA 1 and ss IPCA 2. IPCA1 score and IPCA2 score were the first and second IPCA scores for each genotype from the AMMI analysis. The stable genotypes across the environments were indicated by a small ASV value.

The genotype stability index (GSI) for the stevia genotypes was calculated based on the ASV Rank (RASV) from the genotypes, and the yield performance ranks (RGM) of the genotypes tested in three environments with the following equation:

$$GSI = RASV + RGM$$

The stevia genotypes yield estimation used the GGE biplot analysis according to Yan (2013) with the following formula:

$$\bar{Y}_{ef} - \mu_e - \beta_f = \sum_{k=1}^{t}\lambda_g\alpha_{eg}\gamma_{fh} + \varepsilon_{ef}$$

Where:
$\bar{Y}_{ef}$, μ_e, β_f, k, λ_g, α_{eg}, γ_{fh}, and ε_{ef} are the performance in location 'f' from genotype 'e', overall average yield, the influence of location 'f', number of primer components, the singular value from primer component 'g', the value of genotype 'e' and location 'f' for primer component 'g', and the error of the genotype 'e' in location 'f', respectively.

For the combined ANOVA, AMMI, and ASV, the Genstat 12th software (Goedhart and Thissen, 2009) and the GGE biplot online software PBstat.com (Suwarno *et al.*, 2008) were used.

RESULTS

Genotype by environment interaction (GEI) effects on genotype yield

The results of the homogeneity test showed that the value of Chi square (X^2_h) (74.81) < the value of X^2_t (113.15). This indicates that the leaf yield of stevia in the three environments is homogeneous. Thus, it is necessary to test the combined analysis of variance (ANOVA) to determine the presence of GEI or not.

The combined analysis of variance enunciated that genotype (G), environment (E), and GEI have a significant effect on stevia genotype yield with contributions of 2.43%, 92.38%, and 5.20%, respectively (Table 3). The results further showed that interaction principal component analysis-1 (IPCA-1) has a significant effect with a contribution of 91.33%, while IPCA-2 has no significant impact on the variations caused by stevia genotypes yield, so in this case, the AMMI-1 biplot analysis was applied.

For the stevia genotype yield, Figure 1 shows the box plots analysis in the three environments. At the location, Garut, the recorded stevia genotypes revealed the highest average leaf yield compared with Bandung and Sumedang, West Jawa, Indonesia. The stevia genotypes in Garut also showed wider diversity.

Table 3. Combined ANOVA of the stevia genotypes in three environments of West Java, Indonesia.

Source	d.f.	S.S.	M.S.	F	F-prob	Contribution to total variation (%)
Total	278	790849	2845	*	*	
Treatments	92	414114	4501	20.63	0.00	
Genotypes	30	10056	335	1.54	0.04	2.43
Environments	2	382541	191271	3.40	0.03	92.38
Block	6	337470	56245	257.84	0.00	
Interactions	60	21517	359	1.64	0.01	5.20
IPCA-1	31	19652	634	2.91	0.00	91.33
IPCA-2	29	1865	64	0.29	0.99	8.67
Error	180	39265	218	*	*	
Min. (kg)		2.80				
Max. (kg)		254.67				
Mean (kg)		46.70				
St. Dev.		53.24				
CV (%)		27.73				

d.f. = Degree freedom, SS = Sum of square, MS = Mean square, IPCA = Interaction principal component analysis, Min. = minimal, Max. = Maximal, St. Dev. = Standard deviation, CV = Coeficient of variation

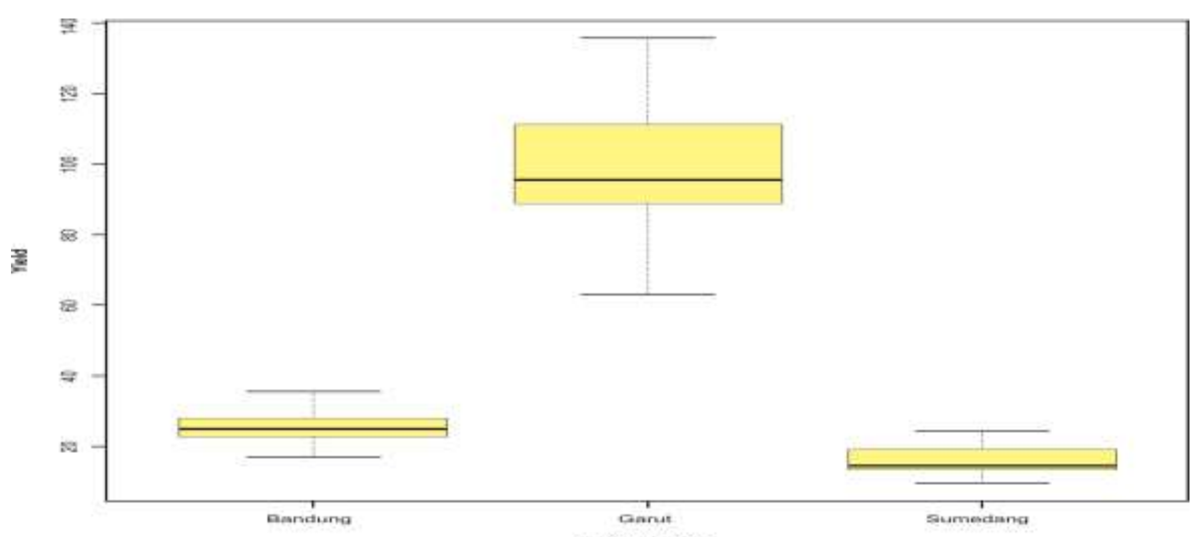

Figure 1. Combined analysis of boxplot for stevia yield in three environments of West Java, Indonesia.

Table 4. Yield potential of the stevia genotypes in each environment of West Java, Indonesia.

Genotypes	Bandung	Sumedang	Garut
G1	18.19 b	9.84 d	95.49 a-g
G2	31.18 ab	12.94 a-d	97.98 a-g
G3	29.01 ab	21.43 a-c	93.34 a-g
G4	27.36 ab	24.27 a	84.71 c-g
G5	25.14 ab	20.47 a-c	97.22 a-g
G6	26.28 ab	13.21 a-d	82.66 c-g
G7	24.37 ab	13.77 a-d	118.15 a-e
G8	21.94 ab	13.72 a-d	86.96 b-g
G9	25.18 ab	13.63 a-d	91.98 a-g
G10	17.16 b	19.21 a-c	74.66 e-g
G11	31.11 ab	19.25 a-c	102.76 a-g
G12	35.53 ab	20.38 ab	69.60 fg
G13	23.37 ab	14.81 a-d	125.86 a-c
G14	20.84 b	15.84 a-d	107.22 a-g
G15	39.71 a	14.53 a-d	114.82 a-f
G16	29.35 ab	9.67 b-d	89.96 a-g
G17	20.79 b	13.41 a-d	63.05 g
G18	23.62 ab	14.12 a-d	121.37 a-d
G19	23.52 ab	18.98 a-c	112.40 a-f
G20	35.02 ab	14.87 a-d	109.58 a-f
G21	24.32 ab	11.06 cd	94.53 a-g
G22	33.58 ab	10.81 b-d	92.03 a-g
G23	25.26 ab	9.93 b-d	135.67 a
G24	23.49 ab	12.96 a-d	131.25 ab
G25	20.88 b	22.82 a	98.17 a-g
G26	25.99 ab	14.28 a-d	117.82 a-e
G27	26.46 ab	19.37 a-c	102.68 a-g
G28	23.02 ab	14.78 a-d	79.02 d-g
G29	19.04 b	13.99 a-d	90.63 a-g
G30	22.40 ab	19.65 a-c	91.56 a-g
G31	26.54 ab	15.01 a-d	87.74 b-g
Means	25.80	15.58	98.74
$LSD_{0.05}$	15.29	1.14	37.84
CV (%)	11.67	17.91	23.47

LSD = Least significant difference; CV = Coefficient of variation, Means followed by the same letter(s) were not significantly different at *P*<0.05.

Leaf yield potential of the stevia genotypes

Table 4 presents the stevia genotypes with leaf yield potential in three different environments. The yield differences of each genotype in each environment based on Duncan's test showed that each genotype has a different potential. At the location, Garut, the genotype G23 leads the rest with the highest leaf yield (135.67 kg), followed by the genotypes G24, G13, G18, G7, G26, G15, G19, G20, G14, G11, G27, G2, G5, G1, G21, G3, G25, G22, G9, G30, G29, and G16. In Bandung, the genotype G15 gave the highest leaf yield (39.71 kg), followed by the genotypes G2, G3, G4, G5, G6, G7, G8, G9, G11, G12, G13, G16, G18, G19, G20, G21, G22, G23, G24, G26, G27, G28, G30, and G31. As for location, Sumedang, genotype G4 showed the highest yield of 24.27 kg followed by genotypes G2, G3, G5, G6, G7, G8, G9, G10, G11, G12, G13, G14, G15, G17, G18, G19, G20, G24, G25, G26, G27, G28, G29, G30, and G31. In stevia genotype leaf yields, the highest variation recorded, especially at Garut, are the genotypes also showing the highest response to the environment, followed by two other environments. In addition, the environmental influence displayed very high leaf yield variations in each environment.

Genotypes yield stability based on AMMI, ASV, and GSI

The AMMI biplot analysis for stevia genotypes yield showed the existence of GEI as recorded and shown by the AMMI-1 analysis in three environments (Figure 2). Interaction principal component analysis (IPCA-1) detailed 91.33% of the stevia genotypes variation through environmental interactions. It implies that the stevia genotype interactions with the three environments in West Java were predicted by the PC-1 of the genotypes and the

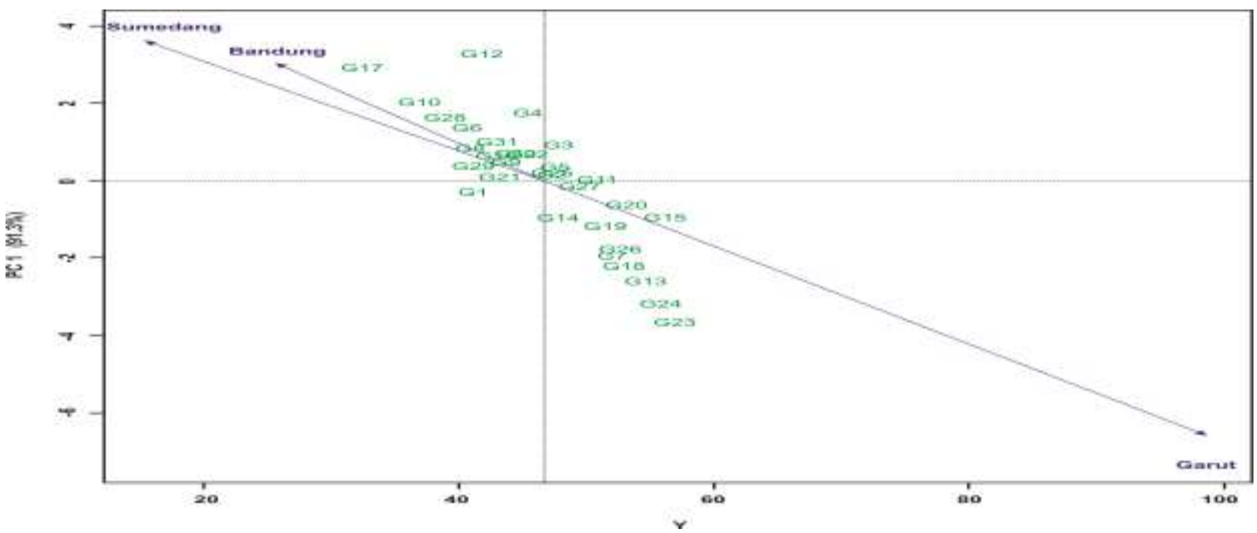

Figure 2. AMMI-1 biplot of the stevia genotypes in three environments of West Java, Indonesia.

environment. The AMMI-1 biplot using the IPCA-1 scores of the yield from the tested stevia genotypes provided the differences among the genotypes in terms of position and length of the yield (X-line) and IPCA-1 score (Y-line) (Figure 2). In the biplot, the genotype and/or environment that was to the right side of the vertical line has a higher average yield than those on the left. The horizontal line shows the stability of the genotypes, and if the genotype position sets close to that line, then it was declared stable.

The stevia genotypes in the right side position include G2, G3, G5, G11, G27, G20, G15, G14, G19, G26, G7, G18, G13, G23, and G24, while the rest placed on the left side of the stability line. The position of the stevia genotypes G11, G27, G2, and G5 sets close to the stability line (horizontal), which indicates that these genotypes had the highest and most stable leaf yield. In addition, Figure 2 also showed that the environment/location, Garut, placed on the right of the stability line, and the stevia genotypes at that location showed the highest average leaf yield than the other two locations.

Table 5 presents the information about AMMI stability value (ASV) and genotype stability index (GSI) display at. According to ASV, the most stable genotype is the stevia genotype G11, followed by five other genotypes, G21, G27, G1, G2, and G5. For GSI measurements, the stevia genotype G11 as also showed the most stable, followed by five other genotypes, G27, G20, G2, G5, and G15.

Stevia yield stability based on GGE biplot

The yield stability analysis of the stevia genotypes, using a GGE biplot, projects in Figures 3, 4, and 5. The results of the PC's value gave 95.8%, indicating the said analysis resulted in a very high contribution to the stevia genotypes in the three planting environments. Figure 3 shows the display pattern “means vs. stability” of the GGE biplot. There were 14 genotypes located on the right side of the Y-axis, namely, G14, G19, G7, G26, G18, G13, G24, G23, G27, G22, G2, G11, G20, and G15. The remaining 17 genotypes are plottedon the left of the Y-axis, which means having less yield and are below the average. Agronomically, the stevia genotypes G27, G2, G11, G20, and G26, resulted as the most stable and had above-average yields. The genotype G20 displayed having a position with less distance to the ideal point, which means that this genotype can produce high yields under optimal and marginal environments.

In the 'representative vs. discriminative' of the GGE biplot, the locations Garut and Bandung have longer vectors than the location, Sumedang (Figure 4). The location, Bandung, can be used as a suitable and representative environment for testing and selecting superior stevia genotypes. Garut has a longer vector but has a larger angle so that it can be used for selecting specific genotypes adaptive to specific environments. Meanwhile, the location Sumedang proves an unsuitable environment for testing the stevia genotypes because it has the shortest vector.

Table 5. Grand mean (yield), AMMI stability value (ASV), and genotypes stability index (GSI) of the stevia genotypes in three environments of West Java, Indonesia.

Genotypes	GM	RGM	IPCAg[1]	IPCAg[2]	ASV	RASV	GSI	RGSI
G1	41.17	26	-0.30	0.29	1.03	4	30	13
G2	47.37	15	0.16	-1.15	1.27	5	20	4
G3	47.93	13	0.91	0.29	2.97	15	28	9
G4	45.45	18	1.76	0.85	5.79	22	40	25
G5	47.61	14	0.35	0.75	1.37	6	20	5
G6	40.72	28	1.36	-0.53	4.45	20	48	27
G7	52.10	8	-1.91	0.11	6.19	24	32	18
G8	40.87	27	0.82	0.21	2.66	13	40	26
G9	43.60	20	0.49	-0.23	1.61	8	28	10
G10	37.01	30	2.00	1.56	6.69	25	55	30
G11	51.04	10	0.04	-0.24	0.26	1	11	1
G12	41.84	24	3.28	-0.99	10.70	30	54	29
G13	54.68	4	-2.60	0.46	8.44	27	31	16
G14	47.97	12	-0.96	0.82	3.23	16	28	11
G15	56.35	2	-0.93	-2.04	3.65	18	20	6
G16	42.99	23	0.65	-1.40	2.54	12	35	23
G17	32.41	31	2.92	0.14	9.48	28	59	31
G18	53.04	6	-2.21	0.30	7.19	26	32	19
G19	51.63	9	-1.17	0.91	3.89	19	28	12
G20	53.16	5	-0.64	-1.35	2.47	10	15	3
G21	43.30	21	0.10	-0.44	0.54	2	23	7
G22	45.47	17	0.70	-1.84	2.91	14	31	17
G23	56.96	1	-3.65	-0.40	11.85	31	32	20
G24	55.90	3	-3.17	0.24	10.30	29	32	21
G25	47.29	16	0.20	1.71	1.83	9	25	8
G26	52.69	7	-1.79	-0.05	5.80	23	30	14
G27	49.50	11	-0.14	0.46	0.65	3	14	2
G28	38.94	29	1.63	0.13	5.30	21	50	28
G29	41.22	25	0.38	0.70	1.41	7	32	22
G30	44.54	19	0.71	1.00	2.51	11	30	15
G31	43.10	22	1.00	-0.27	3.26	17	39	24
Bandung	25.80		3.36	-3.63				
Sumedang	15.58		3.98	3.43				
Garut	98.74		-7.34	0.20				

GM = Grand mean, RGM = Rank of grand mean, IPCAg = Interaction principal component analysis genotypes, ASV = AMMI stability value, RASV = Rank of ASV, GSI = Genotype stability index, RGSI = Rank of GSI

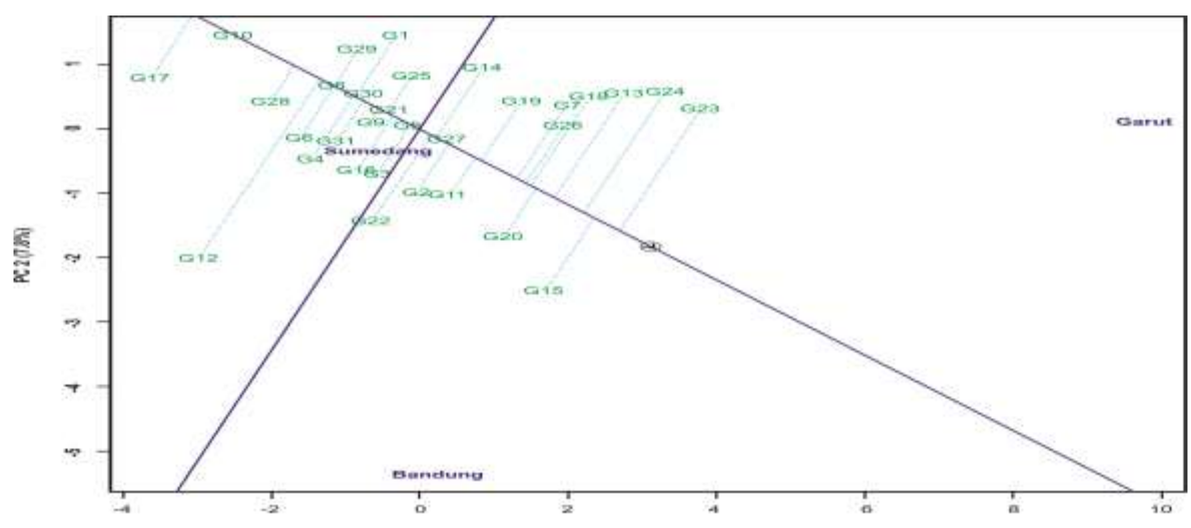

Figure 3. GGE Biplot "means vs. stability" of the stevia genotypes in three environments of West Java, Indonesia.

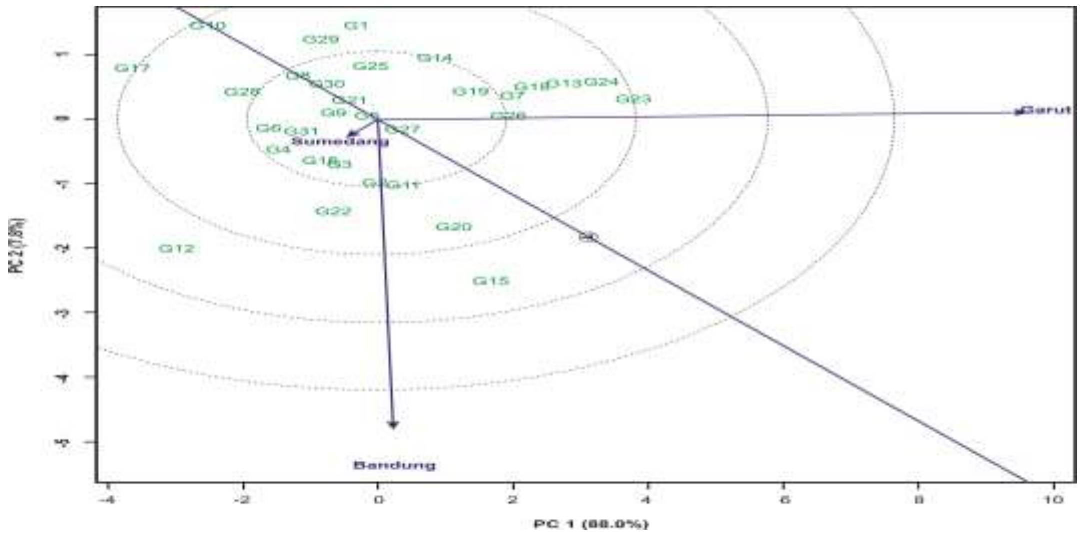

Figure 4. GGE Biplot "representativeness vs. discriminativeness" of the stevia genotypes in three environments of West Java, Indonesia.

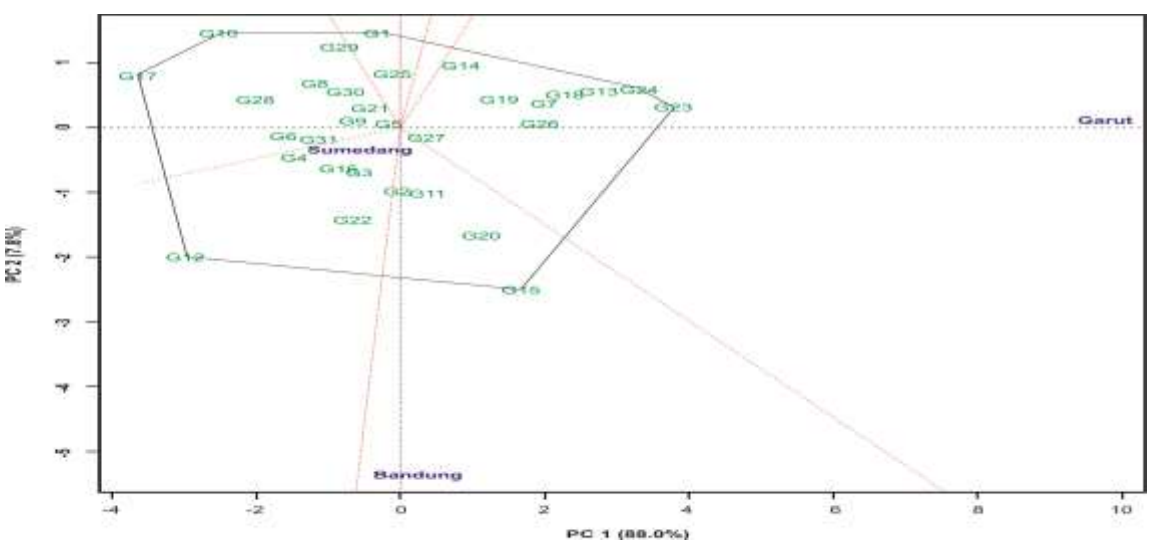

Figure 5. GGE Biplot "which-won-where" of 31 stevia genotypes in three environments of West Java, Indonesia.

The "which-won-where" GGE biplot showed that the environments got divided into seven sectors (Figure 5). The G23 leads all genotypes at Garut, followed by genotype G12 at Sumedang, and genotype G15 at location Bandung. These genotypes had the highest leaf yield in their respective environments. Stevia genotypes G5, G9, G21, and G27 showed the most stable genotypes because of their approach to the sector axis point.

DISCUSSION

The combined analysis of variance showed the significant ($p<0.05$) effects of environments, genotypes, and GE interactions on stevia genotype yield in three environments of West Java, Indonesia. Environmental factors had the highest effects (92.38%), followed by GEI (5.20%) and genotypes (2.43%). The highest variation contributed by environments indicates that the test locations and environments varied widely. A smaller GEI and the large environmental effect implied that the stable stevia genotypes across the experimental environment and the maximum variation in leaf yield resulted from environmental differences. The low genotype effect in this analysis revealed possible because the genotype used has been pre-selected according to commercial needs. However, the genotypic response in the tested environment varied, as indicated by GEI. Past studies also reported that the contribution of test environments was much greater than the genotypes and genotype-by-environment interactions in the multi-environmental experiments (Kumar *et al*., 2014). Other studies also reported that the geological and climate factors strongly influenced the quality and quantity of stevia leaves (Andolfi *et al*., 2006; Benhmimou *et al*., 2017; Khiraoui *et al*., 2021). In addition, the significant GEI in yield assay validates the use of AMMI and GGE biplot analyses to decipher them and determine the yield potential and stability of the evaluated stevia genotypes. Several researchers in the selection of the genotypes also used various stability measures to describe the GEI effects (Karuniawan *et al*., 2021; Maulana *et al*., 2022; Ruswandi *et al*., 2022). Thus, the effect of GEI needs deciphering to determine the stability of the stevia genotypes in three environments in West Java, Indonesia.

The yield variation in stevia genotypes in each environment indicated that the field conditions varied much. Although the temperature was relatively uniform in all the environments during the production period, the rainfall and altitude varied considerably in each environment. Besides, the soil parameters also have significant differences in the locations. These three factors could be the main causes for yield variations in the stevia genotypes across the environments. The average yield performance of each stevia genotype at the location, Garut was relatively higher than at the two other locations, Bandung and Sumedang. It can be due to the high temperature, humidity, altitude, and rainfall patterns shown by the three environments. Bandung has almost the same altitude as Garut, but has lower temperature and lower humidity than Garut. The same data was also reported by Andolfi *et al*. (2006), where stevia has a higher leaf yield in the summer. In case of a low genotype effect, mutation breeding should be conducted in more materials. Moreover, new germplasm from diverse sources should be crossed to these materials or other materials. With low GEI, a few selected locations would be enough for yield testing. With high environment effect (E), high-yielding locations should be selected for commercial production. It suggests that a significant GEI plays a vital role in the breeding of stevia adaptable to a wide range of environments. This interaction gained validation by significant differences in the environmental factors. Thus, the selection of stevia genotypes with high yield may likely be less stable than the averages of all the tested genotypes, requiring only a few representative locations for multi-location trails.

The combined analysis of variance also showed that IPCA-1 had a significant effect, with a contribution of 91.33% to the total variation (Table 3). IPCA-2 have no significant effect on the yield variations, thus, needing the use of AMMI-1 biplot analysis. These findings aligned with the research of Tolorunse *et al*. (2018), which used the AMMI-1 biplot analysis with an IPCA-1 contribution of 69.95% of the total variation in soybean genotypes. In the AMMI-1 biplot, the 15 stevia genotypes showed to be on the right of the vertical line (Figure 2). According to Maulana *et al*. (2020), the orange-fleshed sweet potato genotypes on the right of the vertical line have a higher yield than the overall mean, while genotypes closer to the horizontal line showed a lesser GEI effect and were found stable.

In the latest study, four stevia genotypes (G11, G27, G2, and G5) exhibited high leaf yields showing stability based on the AMMI-1 biplot analysis. In the above four stevia genotypes, three genotypes were mutants (G2 and G5 were the EMS mutants; G11 was obtained through gamma radiation), while G27 resulted as the hybrid of Garut × Bogor-3. According to Gauch (2013), the AMMI stability value (ASV) in the AMMI biplot analysis can provide information about the genotype's stability in multilocation testing. Maulana *et al*. (2020) also added a measurement of the genotype stability index to strengthen the deliberation about the stability of orange-fleshed sweet potato genotypes. The ASV identified the stevia genotype G11 as the most stable, followed by genotypes viz., G21, G27, G1, G2, and G5. The GSI measurements also identify genotype G11 as the most stable, followed by five other genotypes, i.e., G27, G20, G2, G5, and G15 (Table 5). The latest study suggests that the two measurements provide consistent results in selecting stable stevia genotypes.

Based on the GGE biplot analysis, PC1 and PC2 explained 88.00% and 7.80% of the total variation, respectively, and collectively contributed 95.80% of the total variation for stevia genotypes yield in three environments of West Java, Indonesia (Figures 3, 4, and 5). The graph of the GGE biplot 'mean vs. stability' showed that 14 stevia genotypes plot on the right side of the Y-line and 17 plots on the left of the Y-line (Figure 3). According to Ruswandi *et al*. (2021), the Y-line showed the mean yield of each maize genotype, and the X-line showed the stability of the yield of tested maize hybrids. Agronomically, the stevia genotypes G27, G2, G11, G20, and G26, proved the most stable and had above-average yields. According to Mustamu *et al*. (2018), a sweet-potato genotype close to the ideal point on the GGE biplot also has the highest yield and stability. The study showed the stevia genotype G20 plots closer to the ideal point, which means that this genotype can produce high leaf yield under optimal and marginal environments.

The GGE biplot graph 'representative vs. discriminative' revealed that the locations Garut and Bandung have longer vectors than location Sumedang (Figure 4). The environments were classified into three types, namely, Type-I, which has a shorter vector providing little information about the tested genotype and should not be used as a test environment; Type-II, which has a long vector and a small angle with the average abscissa environment, ideal for selecting superior genotypes; and Type-III, which has a long and angular vector with the mean abscissa environment, and such an environment could not be used to select the ideal genotype yet helped identify and select the unstable genotypes in durum wheat (Kendal and Şener, 2015) and maize hybrids (Ruswandi *et al*., 2021). The results of the study detailed the location Sumedang as a Type-I environment because of showing the shortest vector; Bandung a Type-II environment (ideal) because of having a long vector and a smaller axis to the average line (abscissa). Bandung reveals as more suitable for testing and selecting the superior stevia genotypes. The location Garut was a Type-III environment because it has a longer environmental vector yet has a larger angle compared with Bandung, hence can be used to select the area-specific stevia genotypes.

The GGE biplot 'which won where' graph showed that the three locations have seven sectors with different winning genotypes (Figure 5). The sweet-potato genotype at the top of the sector, also has the highest yield in the environment and that sector (Mustamu *et al*., 2018; Karuniawan *et al*., 2021). Genotype G23 was the leading and best- performing genotype at Garut, genotype G12 at Sumedang, and genotype G15 at Bandung. Zhang *et al*. (2016) stated that genotypes at the top of each sector were also the adaptive genotypes to certain environments in proso millet. In addition, Ruswandi *et al*. (2022) also added that maize genotypes in the center of the sector have low GEI effects and were found stable. The study identified four stevia genotypes to approach the sector's central point consisting of G5, G9, G21, and G27. These stevia genotypes have smaller GEI effects than other genotypes, however, they have no high yields, therefore, other measurements need utilization to select the stable and high-yielding genotypes. Vaezi *et al*. (2017, 2019) also reported a similar result, stating that the selection of stable and high-yielding barley genotypes requires more stability measures. Therefore, several yield stability measurements need to be used to select the stable and high- yielding stevia genotypes.

In the latest study, AMMI, ASV, GSI, and GGE biplot measurements proved successful in selecting stable and high-yielding stevia genotypes. The stevia genotypes G11, G27, G2, and G5 had a high and stable yield based on the AMMI-1 biplot. The ASV identified the stevia genotypes viz., G11, G21, G27, G1, G2, and G5 as the most stable, while the GSI measurements recognized the genotypes G11, G27, G20, G2, G5, and G15. Meanwhile, the GGE biplot selects the stevia genotypes G27, G2, G11, G20, and G26 as the most stable genotypes. The genotypes selected through four different measurements consisted of G2, G11, and G27. Thus, these three stevia genotypes can serve as new superior stevia genotypes for having the highest and most stable leaf yields in the three environments of West Java, Indonesia.

CONCLUSIONS

The genotypes, environments, and GEI effects have a significant impact on stevia leaf yield with the contribution of 2.43%, 92.38%, and 5.20% to the total variation, respectively. The three selected stevia genotypes had the highest leaf yield and showed stability in the three environments, namely G2 (Tamangwangu EMS mutant number A), G11 (Bogor mutant with gamma radiation), and hybrid G27 (Garut × Bogor-3). Location Bandung proved as an ideal environment for selecting the stable and high-yielding stevia genotypes, while location Garut revealed a suitable environment for selecting the area-specific genotypes.

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