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Proposed new stability indices using AMMI model and GGE BIPLOT approach to assess G×E interaction

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Abstract
Breeding for novel genotypes with high yield and stability is a crucial objective in agriculture. Environmental factors play a significant role in determining a genotype's response, which is commonly known as genotype-by-environment interaction. In this study, a multi-environment experiment with three replications of twenty-nine pearl millet genotypes for one year (2019) was conducted at eight locations in India. The Additive main effects and multiplicative interaction (AMMI) and Genotype and Genotype-by-environment (GGE) biplot analyses were used to study the genotype-by-environment (G x E) interaction and identify stable genotypes. A new weighted stability index was proposed, which was based on standardized grain yield indices and AMMI-based stability parameters to determine high-yielding and stable genotypes. In all environments, seven independent principal component axes (IPCAs) were significant. AMMI-based stability parameters and stability indices were used to identify stable genotypes, while yield stability index and weighted index were employed to identify the most stable and highest-yielding genotypes simultaneously. According to the AMMI-based stability parameters, genotypes G27, G13, and G28 were found to be stable, while genotypes G10, G11, and G13 were identified as stable with high grain yield according to yield stability index and weighted index. These findings suggest that the proposed weighted stability index can be used to identify high-yielding and stable pearl millet genotypes.

Keywords: AMMI and GGE model, stability analysis, yield stability index (YSI), weighted index (WI)

1. Introduction

In India, pearl millet [*Pennisetum glaucum* (L.) R. Br.] is known as bajra and is a highly cross-pollinated crop in a protogynous state. It belongs to the Poaceae family (Animasaum *et al.*, 2019) [1]. It is one of the millets that is frequently cultivated in India in both arid and semi-arid environments, having both food and non-food applications. Nearly 90% of acres in the country's drier regions, mostly in the states of Rajasthan, Haryana, UP, Gujarat, and Maharashtra, are used to grow pearl millet making it India's fourth most popular staple crop after rice, wheat, and maize.

The complex nature of grain yield is influenced by polygenes and environmental factors. Thus, understanding these interactions could have a significant impact on future research for yield improvement and the selection of varieties for specific environments (Nyadanu and Dikera, 2014) [16]. In multilocation varietal yield experiments, the AMMI model has been recommended as a superior alternative approach for analysing genotypes by environment interaction (Gauch, 1993) [6]. The genotype-by-environment interaction refers to the variation in how a genotype responds to different environments. In this context, a genotype is considered stable if it responds to the environment in a way consistent with the mean response of all genotypes. Supporting the AMMI analysis for selecting high-yielding genotypes with dynamic stability are different AMMI stability measures and the yield stability index (YSI).

A genotype x environment dataset's genotypic main effect (G) and genotype x environment (GxE) interaction are shown in a biplot known as a GGE biplot (Yan *et al.*, 2000). When genotype by environment two-way data was evaluated, a technique called GGE biplot analysis is used to fulfill diverse research goals. It consists of a series of biplot graphs. With high and the greatest stability, this technology could aid the breeder in understanding the G x E interaction effect and selecting the best genotypes for various environments (Farshadfar 2008; Farshadfar *et al.,* 2011) [5-8].

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Karimizadeh *et al.*, (2012) [11] evaluated lentil yield in twelve environments in four environments for three years in Iran using wricke ecovalence and AMMI parameters including SIPC, ASV, MASV, and D1 and found positive significant correlation with multi year results. Kumar *et al.*, (2018) [13] assessed nineteen genotypes of barley grown at eight different locations to determine stable genotypes with diverse stability using AMMI and GGE biplot models with the ranking pattern of Yield stability index and AMMI stability value. Using the AMMI and GGE biplot analysis, the G x E interaction of pearl millet genotypes was evaluated (Mamata *et al.*, 2019) [15]. Verma and Singh (2020) [19] evaluated the stability of wheat genotypes for years across the Peninsular zone of the country for two years using a weighted average of an absolute score, AMMI stability value, ASV, MASV and ASTAB measures. Karuniawan *et al.* (2021) [12] determined the stable genotypes of sweet potato under three environments in Indonesia depending upon AMMI, AMMI stability value, and GGE Biplot models.

The current study assessed genotype and environment interaction in pearl millet genotypes from eight distinct regions in India, utilizing AMMI and GGE biplot analysis methodologies. The study identified the most stable genotypes using the AMMI-based stability parameter (ASTAB) and the Stability Index. Additionally, the study identified the most stable and highly productive genotypes using the indices YSI and WI. A new weighted index (WI) was suggested as a means of finding stable and high-yielding genotypes, primarily based on the normalized grain yield and ASTAB indices.

2. Materials and Methods
2.1 Data Set
A multi-environment experiment including 29 genotypes (Table 1) of pearl millet was carried out over the period of one year, in 2019, at eight locations in India: ARS Mandor, Bikaner, RARI Jaipur, Jamnagar, Hisar, Gwalior, Ranchi, and Jammu Kashmir with three replications in RBD. The data were recorded from Agricultural University, Mandor, Jodhpur, Rajasthan India.

Table 1: List of twenty-nine genotypes evaluated at eight environments in India

Sr. No.	Genotype Code	Genotypes	Sr. No.	Genotype Code	Genotypes
1	G1	HHB272	16	G16	HHB223
2	G2	MPMH21	17	G17	GHB744
3	G3	RHB177	18	G18	GHB732
4	G4	HHB197	19	G19	KBH108
5	G5	GHB538	20	G20	86M86
6	G6	HHB67	21	G21	Kaveri Super Boss
7	G7	AHB1269	22	G22	MP-7792
8	G8	HHB299	23	G23	Proagro9444
9	G9	AHB1200	24	G24	GHB558
10	G10	PB1705	25	G25	Dhanshakti
11	G11	XMT1497	26	G26	ICMV221
12	G12	86M01	27	G27	PusaComposite701
13	G13	GHB905	28	G28	Posa Composite383
14	G14	MPMH17	29	G29	JBV 2
15	G15	RHB173			

2.2 AMMI and GGE Biplot Model
The term "AMMI analysis" refers to the integration of multiplicative effect analysis and analysis of variance (ANOVA). The model proposed by Rao and Prabhakaran (2005) of AMMI is as follows.

$$Y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{n} \lambda_n \alpha_{in} \gamma_{jn} + \theta_{ij} \qquad (1)$$

$$\theta_{ij} \sim N(0, \sigma^2); i = 1, 2, \dots, G; j = 1, 2, \dots, E$$

Where,
Y_{ij} = mean yield of ith genotype in j^{th} environment/location
μ = general mean
g_i = i^{th} genotypic effect
e_j = j^{th} location effect
λ_n = eigen value of the IPCA axis n
α_{in} and γ_{jn} are the ith genotype j^{th} environment PCA scores for the axis n
θ_{ij} = residual
The residual combines the PCA scores from the N - n' discarded axes, where N = min (T-1, S-1). The additional restrictions in model (I) are

$$\sum_{i=1}^{T} \alpha_{in}^2 = \sum_{j=1}^{S} \gamma_{in}^2 = 1 \,\forall\, n;$$

$$\sum_{i=1}^{T} \alpha_{in} \alpha_{in}^* = \sum_{j=1}^{S} \gamma_{jn} \gamma_{jn}^* = 0, n \neq n^*$$

and $\lambda 1 > \lambda 2 > \cdots \dots \dots > \lambda n > 0$

Testing the mean square of each axis with the estimated residual using F-statistics determines the number of PCA axes to be preserved in many real cases (Gollob, 1968; Gauch, 1988) [7, 8].
By using single value decomposition (SVD) in a rank matrix, data matrix can be optimally approximated. The fundamental framework for creating a GGE biplot using GE data is

$$Y_{ij} = \mu + g_i + e_j + \emptyset_{ij} + \theta_{ij} \qquad (2)$$

Where, $\emptyset_{ij}$ is the interaction between g_i and e_j, θ_{ij} is residual associated with genotype *i* in environment *j*.
Weighted Stability Index (WSI): The AMMI model lacks the inclusion of a quantitative stability measure required for the categorization and ranking of genotypes based on their yield stability. The AMMI based stability parameter (ASTAB) was discovered by Rao and Prabhakaran as follows:

$$\text{ASTAB} = \sum_{n=1}^{N} \lambda_n \gamma_{in}^2 \qquad (3)$$

Where, λ_n and γ_{in}^2 are the eigenvalues of the nth IPCA and the eigenvector values for the ith genotype. Smaller the value of the statistic, more stable a genotype is thought to be.

According to the following formula proposed by Babarmanzoor *et al.* (2009) [2], sustainability index (SI) was calculated as

$$SI = \left[\frac{\bar{Y}_i - s_i}{\max(Y_{i1}, Y_{i2}, \ldots Y_{is})}\right] X\ 100 \qquad (4)$$

Where, $\bar{Y}_i$ is the average of ith genotype, s_i is the standard deviation, $\max(Y_{i1}, Y_{i2}, \ldots Y_{is})$ the value of good genotype across any environment. The following five categories for genotype classification are based on SI values:

SI value	Stability
Upto 20%	Very Low
21 - 40%	Low
41 – 60%	Moderate
61 – 80%	High
above 80%	Very High

The yield stability index (YSI) is a metric for determining stable and high-yielding genotypes (Oliveira *et al.*, 2014) [17], is given as

$$YSI = R(ASTAB)_i + R(GY)_i \qquad (5)$$

Where, $R(ASTAB)_i$, $R(GY)_i$ are the rank of AMMI based stability parameter and mean grain yield of ith genotype in environments. The Yield Stability Index (YSI) is a single criterion that combines mean yield and stability. Desirable genotypes with high mean yield and stability are those with low values of the YSI parameter.

The normalized index by Hooda *et al.* (2017) [9] of ith genotype for all the environments can be generated as

$$NGY_i = \frac{GY_i - Min(GY_i)}{Max(GY_i) - Min(GY_i)}$$

Where, GY_i is the the mean grain yield of the ith genotype across all the environments and $Max(GY_i)$ and $Min(GY_i)$ are derived for i^{th} genotype.

Additionally, if $ASTAB_i$ is the magnitude of AMMI based stability parameter of ith genotype for all the environments where lower the ASTAB more stable in the genotype then normalized index (Hooda *et al.* 2017) [9] of ith genotype for all the environments can be written as:

$$NASTAB_i = \frac{Max(ASTAB_i) - ASTAB_i}{Max(ASTAB_i) - Min(ASTAB_i)}$$

Where, $NASTAB_i$ is the normalized index of AMMI based stability parameter, where NASTAB value is higher, the genotype is more stable. The normalized value of indices ranges from zero to one, increasing or decreasing in the stability direction, where lower values denote lower stability and higher values denote better stability.

We suggest the weighted stability index(WSI) which identifying the stable genotype of high yield through normalized indices of grain yield and ASTAB

$$WSI_i = W_1 NGY_i + W_2 NASTAB_i, \text{ i= 1, 2,.....G} \qquad (6)$$

Here, $(0 \le W_1, W_2 \le 1$ and $W_1 + W_2 = 1)$ are weights related to NGY and NASTAB, where W_1 and W_2 are follows as

$$W_1 = \frac{s_2}{s_1 + s_2} \text{ and } W_2 = \frac{s_1}{s_1 + s_2}$$

Where, s_1, s_2 are the standard deviation of NGY_i and $NASTAB_i$ respectively. The weighted index ranges from 0 to 1. For genotype stability, a straightforward ranking of genotypes based on WI was adopted. To establish the comparability of conclusions derived from the suggested index YSI, Spearman's rank correlation coefficient was employed. This was done by generating the rank-based Yield Stability Value (YSI) and Weighted Index (WI), where the genotype with the highest WI index represents the most stable and high-yielding genotype. The use of Mamata and Hooda's (2020) [14] weighted yield and stability indices enabled the ranking of genotypes based on the computation of all stability parameters in the AMMI model.

The R software with the "metan" and "GGEbiplot2" packages were utilized for conducting the statistical analysis of the AMMI model and GGE biplot in the current study. The AMMI model and GGE biplot allowed for the estimation of various stability statistics and the construction of the model.

3. Results and Discussion
The ANOVA using AMMI revealed that the grain yield of pearl millet was significantly influenced by both environment and genotype, as well as their interaction ($p < 0.01$). The environmental factor accounted for the highest proportion of variance (40.16%), followed by the G x E interaction (20.13%) and genotypic variation (11.79%). The dynamic nature of the environments, attributed to factors such as temperature and precipitation patterns, as well as biotic and abiotic variables, explained a large portion of the variation across environments (Zewdu *et al.,* 2020) [23]. The first seven principal component axes of the AMMI model, which represented 40.69%, 18.36%, 15.39%, 10.75%, 6.30%, 5.73%, and 2.88% of the total variation, were significant in explaining G x E interaction. The significant amount of GEI justified the estimation of genotypic stability across environments.

Table 2: AMMI ANOVA of pearl millet genotypes of grain yield (kg/net plot)

Sources of variation	DF	SS	MSS	F	SS (%)
Environment(E)	7	363.87	51.98	157.13**	40.16
Rep.(Env.)	16	5.29	0.33	2.27**	0.58
Genotype(G)	28	106.84	3.82	26.20**	11.79
Genotype x Environment	196	182.42	0.93	6.39**	20.13
PC1	34	74.23	2.18	14.99**	40.69
PC2	32	33.49	1.05	7.19**	18.36
PC3	30	27.89	0.93	6.38**	15.29
PC4	28	19.61	0.70	4.81**	10.75
PC5	26	11.50	0.44	3.04**	6.30
PC6	24	10.45	0.44	2.99**	5.73
PC7	22	5.26	0.24	1.64**	2.88
Residuals	448	65.24	0.15		
Total	891	906.08	1.02		

AMMI-based stability index (ASTAB)

To address the need for a method that combines mean yield and stability into a single index, various researchers have introduced different selection criteria (Kang, 1993; Dashiell, 1994; Babarmanzoor *et al.,* 2009) [10, 3, 2]. In this context, the ASTAB accounts for a number of IPCAs that explain the majority of the variation in the GE interaction. The rank of ASTAB and yield mean were set up so that the lowest ASTAB takes the top spot and the highest yield mean takes the top spot. The ranks were then added to create a single simultaneous yield and yield stability index, known as the yield stability index (YSI). The YSI with the lowest grain yield was considered the most stable. Genotypes G28 and G25 were identified as the most reliable, followed by genotype G29, based on stability index (I). Therefore, a single selection index must consider both phenotypic characteristics and stability. Based on the yield stability index (YSI), G10 and G11 were identified as the most stable genotypes with high grain yield. According to ASTAB, G27 and G13 genotypes were regarded as more stable, with lower values of this statistic.

Based on the weighted index (WI), the stable genotypes with higher grain yield were found to be G10, G11, and G19. The sustainability index (SI) showed that only two sets of stable genotypes were identified. Genotypes G1, G3, G4, G5, G6, G7, G9, G12, G13, G14, G16, G21, G22, G23, G24, G26, G28, and G29 had very low SI percent compared to G2, G8, G10, G11, G15, G17, G18, G19, G20, G25, and G27, which had low SI percent. It is essential to note that the YSI, WI, and SI provide different perspectives on yield stability, and therefore, the use of multiple indices can aid in selecting the most stable and high-yielding genotypes.

The Spearman's rank correlation coefficient of 0.957 between the yield stability index (YSI) and weighted stability index (WSI) was significant at the 1% level of significance based on a student t-test. This indicates that both indices perform nearly equally well in identifying stable genotypes with high yields. These results were consistent with a report by Zali *et al.* (2012) [22] in which thirteen different stability measures were considered.

Table 3: Yield- stability indices for eight- environments of India

Genotypes	GY	ASTAB	YSI	I	WI	SI(%)	SIG
G1	2.582	0.332	33	0.331	0.63	9.778	very low
G2	2.704	0.307	26	0.216	0.68	30.259	low
G3	2.796	0.356	25	0.144	0.69	0.661	very low
G4	2.798	0.699	34	0.745	0.59	3.454	very low
G5	2.690	0.576	36	0.409	0.59	17.349	very low
G6	2.605	0.472	35	0.522	0.60	17.540	very low
G7	2.774	0.548	32	1.530	0.62	13.752	very low
G8	2.949	0.678	30	0.560	0.64	20.747	low
G9	2.313	0.903	49	0.299	0.38	10.847	very low
G10	3.530	0.536	15	0.380	0.86	27.524	low
G11	3.423	0.452	15	0.476	0.85	21.661	low
G12	3.097	0.798	31	0.153	0.65	3.507	very low
G13	2.897	0.194	16	0.205	0.77	0.319	very low
G14	3.300	0.992	31	0.255	0.65	19.430	very low
G15	3.153	1.244	38	0.236	0.52	24.657	low
G16	3.010	0.841	33	0.170	0.61	12.684	very low
G17	2.678	0.998	48	0.166	0.46	26.457	low
G18	3.215	1.085	35	0.340	0.59	24.360	low
G19	3.464	0.720	22	0.296	0.78	40.93	very low
G20	3.160	0.661	25	0.533	0.71	23.540	low
G21	3.359	1.930	35	0.150	0.38	12.239	very low
G22	3.576	0.979	24	0.147	0.73	18.652	very low
G23	3.431	1.037	30	0.213	0.67	14.923	very low
G24	2.601	0.267	30	0.150	0.66	-9.444	very low
G25	2.125	0.400	37	0.104	0.47	-30.442	low
G26	2.118	0.234	33	0.201	0.52	9.208	very low
G27	2.739	0.119	20	0.211	0.75	22.023	low
G28	2.826	0.208	18	0.080	0.75	-18.153	very low
G29	2.688	0.503	34	0.125	0.61	0.641	very low

Visualization of genotype stability and high grain yield in various environments

The scatter plot of NGY along the x-axis and ASTAB along the y-axis is shown in Figure 1. This scatter plot provides a quick overview of the genotypes that are both high-yielding and stable. The genotypes that appear in the top-right quadrant of the scatter plot are considered to be the most desirable, as they are both high-yielding and stable. In this study, G27 was identified as the most stable genotype based on ASTAB, while G22 was found to be high-yielding based on NGY. The genotypes G10 and G11 were identified as the most desirable, as they were both high-yielding and stable according to both indices.

Fig 1: Visualization of genotype stability and high grain yield

The three key factors of GGE biplots for pearl millet genotypes across environments:
1. Mega-environment analysis using the which-won-where pattern and its genetic association with location.
2. Assessment of the representative and discriminating power of test location.
3. Genotype assessment based on stability and average performance over a mega-environment.

Mega-environment analysis
The "which-won-where" pattern of the multi-environment yield trial data must be visualised in order to examine the potential existence of various mega-environments (ME) in each area. A mega-environment can be defined as a group of environments that consistently share the best set of genotypes among them. The G x E yield information is revealed in Figure 2.

Fig 2: Mega-environments analysis across locations

A polygon was created in the "which-won-where" biplot (Figure 2) between the genotype vector that was furthest from the origin and lines that were drawn perpendicularly from the biplot's origin to each of the polygon's sides. One of the winning genotypes interacts more than the others when the environment deviates from this virtual line, which is the direction in which we lean. By dividing the entire trial region into homogeneous groups according to genotype performance, more information was acquired about the environments as well as genotypes. To ensure that all genotypes fit inside the polygon, an irregular convex polygon has been built. The environments that fall within one of the sectors created by the perpendicular lines perform better in that particular sector, and the genotypes with the best performance are found at the vertices of these sectors. As a

result, the environments were separated into various sectors, each of which had its own winning genotypes. For pearl millet grain yield data, the "which won where" biplot (Figure 2) showed that ARS Mandor (E1), Bikaner (E2), RARI Jaipur (E3), Jamnagar (E4), Hisar (E5), Gwalior (E6) and Jammu Kashmir (E8) were made up one mega environment, and the remaining one Ranchi (E7) made up a second mega environment. The winning genotypes in the corresponding mega settings have been found to be G11 and G21.

Test-environment evaluation
As illustrated in Figure 3, the correlation between the environments was assessed using the cosine of the angle between them. By averaging all environments, the target environment was represented as an arrow on the average

coordination axis (AEC), and the angle between the target and test environments was used to determine each environment's representativeness of the other. The variation of the variable environment revealed the discriminating property.
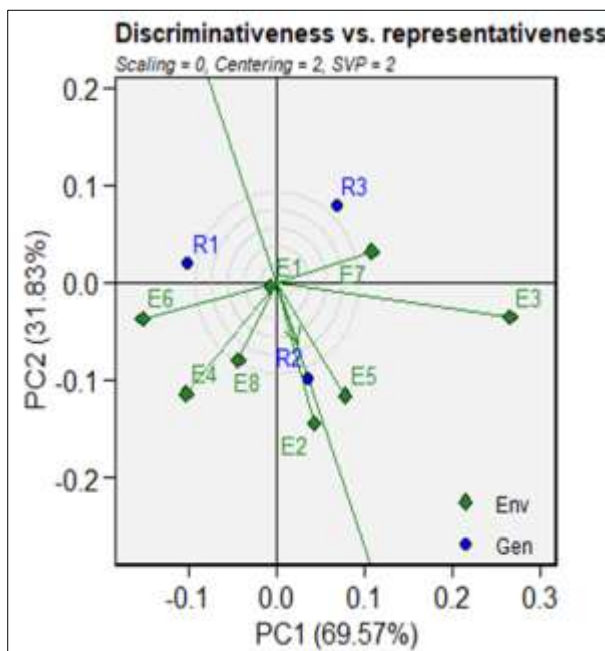

Fig 3: Test-environment evaluation across locations

The cosine of the angle between the two environments is used to calculate the relationship and to depict it as a line linking to the biplot origin (Yan and Tinker, 2006). It was noted that RARI Jaipur (E3) had the strongest discriminating power while Bikaner (E2) had the most trial-wide representativeness. ARS Mandor (E1) exhibited poor discriminating power and low representativeness. The RARI Jaipur (E3) environment was found to be the most fruitful trial, while the ARS Mandor (E1) environment the least fruitful.

Genotype evaluation
The major goals of breeding programs are to improve agricultural output and find superior genotypes. When analysing genotypes, the test environment assessment axis (Figure 4) seems useful. A perpendicular axis was placed on the biplot and is usually known as the average coordination axis (ACA), while the axis running across this virtual environment, is known as the average environment axis (AEA).
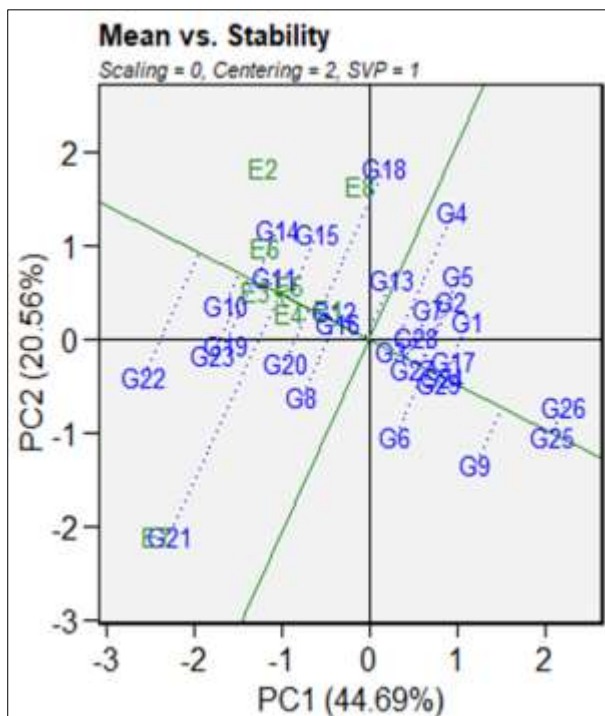

Fig 4: Genotype evaluation across locations/environments

The genotypes are ranked according to mean performance by the arrow that is displayed on the axis of the AEC abscissa, which points in the direction of higher mean performance for the genotypes. The ranking of the genotypes on the AEC abscissa has always been absolutely or strongly associated with G, eliminating cases where the genotypic effect (G) is

too small to be significant. According to Fig. 4, the genotypes G3, G16, G24, G25, G27 and G29 were preferable for the trial region in terms of average yield and genotype stability. In comparison, the least stable genotypes were G18 and G21.

4. Conclusion
As a result, it was determined that the AMMI model was a useful tool for analyzing GEI in pearl millet yield experiments across multiple environments. The stability measures YSI and WSI were observed to have a strong correlation with one another as well as a significant outcome. To find stable, high-yielding genotypes, one may use the parameters YSI, ASTAB, I, WI, and SI. The genotypes G7, G9, and G21 were found to be the least stable according to the Stability index (I), YSI, and ASTAB respectively, with poor yield, while genotypes G10, G11, G13, G27, and G28 were found to be the most stable and high yielders.

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