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Assessment of Genetic Stability in Chickpea Varieties Through GGE and AMMI Analyses

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ABSTRACT

Background: Chickpea is grown in diverse agro-climatic conditions and various varieties respond differently to these environments. Stability analysis identifies adaptable varieties that yield consistently across regions. Our study focuses on identifying high-yield, reliable chickpea varieties through extensive multi-environment trials

Methods: To accomplish our research goal, we conducted a comprehensive stability analysis during 2020-21 and 2021-22 using 12 different methods which include parametric, non-parametric and PCA-based AMMI, GGE biplot analysis. This extensive analysis was applied to six distinct Chickpea varieties cultivated in three diverse environments.

Result: CSJ515 consistently demonstrated stability in all 10 analyses, while GNG2144 and RSG959 showed stability in 7 analyses. GNG1958 exhibited stability in four analyses and GNG2171 and RSG974 were stable in 3 and 2 analyses, respectively. These results suggest that CSJ515, GNG2144 and RSG959 have broad and stable adaptability across diverse environments.

Key words: AMMI, Chickpea stability analysis, G x E interaction, GGE Biplot.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop adapted to arid and semiarid conditions, providing essential protein for both human and animal diets (Bampidis *et al*., 2011). It plays a vital role in western Rajasthan's agriculture, particularly during the rabi season, occupying a significant share in India's pulse crop production on approximately 21.00 lakh hectares (Anonymous, 2021). A plant's phenotype is shaped by its genotype, environment and their interactions (Meena *et al*., 2022). Genotype-environment interactions result in performance variations across environments, crucial for identifying superior varieties (Yan and Kang, 2003). Understanding these interactions is vital in plant breeding and agronomic stability analyses, which encompass parametric and nonparametric methods (Huehn, 1990). Breeders often customize stability analysis techniques, combining both types (Sitaresmi *et al*., 2012). Modern agriculture relies on genetically stable crop varieties, developed to meet global food security needs. However, chickpea variety research lacks comprehensive stability analysis across diverse environments, specifically employing AMMI and GGE biplot methods. Many chickpea varieties exist, yet their performance in varying conditions remains poorly understood. AMMI and GGE analyses can reveal variety-environment interactions, aiding stability assessment and variety selection. Addressing this gap can enhance chickpea breeding strategies, yielding more resilient varieties for improved global food security.

MATERIALS AND METHODS

In this Study, we assessed the six chickpea varieties (CSJ-515, GNG-2144, GNG-2171, GNG-1958, RSG-974 and RSG-959) for their genotypic stability in the climate zone IIB of Rajasthan, India, during the *rabi* seasons of 2020-21 and 2021-22. The evaluation aimed to determine their yield potential and adaptability to the unique environmental conditions of the Luni basin. Multi-environment trials were carefully designed and carried out using a randomized block design, with each plot measuring 4 × 3 m^{2} and a planting arrangement of 30 × 10 cm. The study included three replications and spanned three distinct locations: the Agricultural Research Substation, Sumerpur (E1 and E2) and the Agriculture Technology Centre in Sumerpur (E3). The research assessed various agronomic parameters, including seed yield per plot (SYP), days to 50% flowering (DF), plant height (PH), days to maturity (DM), primary branches per plant (PBP), number of pods per plant (NPP) and Number of Seeds Per Pod (NSP). The data analysis utilized PBSTAT-GE software (www.pbstat.com) to estimate various stability parameters

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like parametric, nonparametric and two PCA-based stability methods *i.e*. AMMI and GGE biplot.

RESULTS AND DISCUSSION

Agronomic performance

The agronomic evaluation of tested varieties revealed significant performance variations. PH ranged from 52 to 61 cm, DF from 65 to 76 days and DM from 116 to 130 days. PBP ranged from 5 to 6.67, indicating diverse yield capacities. NPP varied between 47.67 to 62.67 and NSP showed a narrower variation, 1.56 to 1.89. SYP varied from 1.82 to 2.65 kg. Statistically significant differences were observed in all traits except NSP, emphasizing the importance of considering various growth patterns in variety evaluation (Ramamoorthy *et al*., 2016).

Parametric stability

Francis and Kannenberg (1978) introduced the Coefficient of Variability (CVi) as a key concept in stability analysis. They proposed that a genotype can be considered stable if it consistently yields above the mean yield while maintaining a CVi below the average. Based on CVi we categorized genotypes into four groups. Group I, exemplified by CSJ515, exhibited high grain yields above the mean and low CVi values, signifying enhanced stability. In contrast, Group II included varieties like GNG2171 and GNG2144, which had above-average yields but lower stability compared to Group I. Group III varieties, such as RSG959 and RSG974, had modest yield potential but showed notable stability. Lastly, Group IV, represented by GNG1958, displayed below-average grain yields and lacked stability. In summary, the CVi concept helps identify stable genotypes with above-average yields (Group I), while other groups vary in terms of yield potential and stability.

Genotype stability in plants represents their genetic ability to adapt to varying environmental conditions. Finlay and Wilkinson (1963) introduced the concept of assessing stability through the regression coefficient ‘bi.’ When bi equals 1, a genotype is considered averagely stable, excelling in diverse conditions. Deviations from 1 hold significance: bi < 1 indicates a preference for lower-yield environments, while bi > 1 suggests high-yield adaptability. Eberhart-Russell’s analysis stresses precise stability determination by evaluating linear regression coefficients (bi) and nonlinear components (s^2di) within genotype-environment interactions. A genotype with bi = 1 and s^2di = 0 signifies stability. All tested varieties demonstrated stability except GNG2171 according to Eberhart and Russell (1966) (Table 1).

Wricke (1962) introduced the ecovalence method (Wi^2) as a means of quantifying the relative contribution made by individual genotypes in relation to the cumulative sum of squares representing genotype × environment interactions. Genotypes exhibiting lower than the average ecovalence values are indicative of stability. In the present study, the genotypes demonstrating stability aligning with low ecovalence values were identified as CSJ515 and RSG959 (Table 1).

Hanson (1970) introduced a technique for assessing genotype stability under limited conditions, using the Di parameter to infer stability, with CSJ515 and RSG959 showing enhanced stability. Subsequently, Shukla’s measure of genotype stability variance (σ^2) was applied, with lower variation indicating greater stability. According to Shukla’s (1972) criterion, CSJ515, RSG959 and GNG2144 were identified as stable genotypes, while GNG2171, RSG974 and GNG1958 exhibited higher variability across environments (Table 1).

Nonparametric stability

Kang’s yield and stability index (YSi) integrates both genotype yield and σ^2 into a unified test statistic. Another pertinent nonparametric stability metric is Kang and Pham’s (1991) rank-sum, wherein both yield and σ^2 serve as selection parameters. This metric assigns equal weight to both yield and stability, facilitating the identification of genotypes that are both high-yielding and stable. The genotype with the highest yield receives a rank of 1, while the genotype with the lowest stability variance is also assigned a rank of 1. Cumulative ranks based on yield and stability variance are computed for each genotype, with the smallest cumulative rank-sum indicating preference. Genotypes with YSi values surpassing the mean YSi are considered for selection, including CSJ515, GNG1958, GNG2144, GNG2171 and RSG974 (Table 2).

Nassar and Huehn (1987) introduced two stability methods, $S^{(1)}$ and $S^{(2)}$, based on relative performance rankings across environments. Genotypes with stable rankings exhibit subtle variations, as noted by Becker and Leon (1988). Evaluation using $Zi^{(1)}$ values identified no genotypic instability relative to others, while $Zi^{(2)}$ highlighted three unstable genotypes: GNG1958, GNG2171 and RSG974 (Table 2). The stability parameters $S^{(1)}$ and $S^{(2)}$ are

Table 1: Parametric stability analysis for seed yield.

Genotype	Yi (Kg/Plot)	CVi	bi	P_bi	s^2di	P_s^2di	Wi^2	Di	σ^2
CSJ-515	2.69	6.34	1.55^{ns}	0.293	-0.01^{ns}	0.720	0.01	0.16	0.01
GNG-1958	2.26	8.14	1.44^{ns}	0.389	0.01^{ns}	0.242	0.02	0.21	0.04
GNG-2144	2.37	6.92	1.24^{ns}	0.633	0.00^{ns}	0.260	0.02	0.2	0.03
GNG-2171	2.41	8.61	0.61^{ns}	0.445	0.06 *	0.021	0.08	0.32	0.17
RSG-959	1.77	6.24	0.72^{ns}	0.581	0.00^{ns}	0.342	0.01	0.19	0.02
RSG-974	2.28	4.83	0.44^{ns}	0.282	0.01^{ns}	0.229	0.03	0.21	0.05

measured separately and strongly correlated when using uncorrected data, with almost perfect correlation when data is corrected for genotype effects. In this study, two stability parameters, Si$^{(3)}$ and Si$^{(6)}$, were established to assess yield and stability across different locations, following Huehn's (1979) methodology. Genotypes with Zi values below the Chi-squared table's critical value demonstrated greater stability. CSJ515 and RSH959 exhibited lower Si$^{(3)}$ and Si$^{(6)}$ values, while GNG2171 was the least stable genotype. Fox *et al*. (1990) introduced the "TOP" measure for assessing cultivar adaptability, ranking them at each location. Genotypes consistently in the top third were considered widely adaptable. Following this approach, CSJ515, GNG2144 and GNG2171 showed heightened adaptability, while GNG1958, RSG959 and RSG974 had lower adaptability. Additionally, using Thennarasu's (1995) stability criterion, genotypes with minimal NPi$^{(1)}$, NPi$^{(2)}$, NPi$^{(3)}$ and NPi$^{(4)}$ values were deemed more stable. Based on these NPi values, GNG1958, GNG2144, RSG959 and RSG974 emerged as the more stable genotypes (Table 2).

Correlation between parameters of stability

The examination of Spearman correlations among stability parameters (Table 3) revealed significant associations. Yi showed positive correlations with YSi and TOP, while displaying negative correlations with NPi$^{(2)}$, NPi$^{(3)}$ and NPi$^{(4)}$. A positive correlation between YSi and TOP supported the efficacy of TOP as a stability parameter for identifying high-yielding genotypes (Becker *et al*. 1988). Selecting for yield enhancement may alter grain yield stability through increased TOP, enabling specific genotypes for specific environments. Notably, bi exhibited no substantial correlation with Yi or stability parameters. Genotype CSJ515 had the highest yield and was deemed stable based on prominent stability parameters shown by principal component analysis (Fig 1A). This observation can be explained by TOP's calculation from high-yield ranking locations.

Additive main effects and multiplicative interaction (AMMI)

The AMMI methodology effectively unravels genotype-environment interactions, but validation for site-specific genotypes is essential (Sitaresmi *et al*., 2012). ANOVA for AMMI revealed a significant variation for the environment, genotype and genotype-environment interaction effects. Genotype-environment interaction effect was decomposed into two components, explaining all variability. Biplot analysis, namely AMMI1 and AMMI2 biplots, is valuable for interpreting the AMMI model. AMMI1 depicts yield vs. PC1, while AMMI2 illustrates PC1 vs. PC2 (Suwarto *et al*., 2011).

The AMMI1 biplot illustrates genotype adaptation, emphasizing the well-adapted genotype with a high average yield and consistent interaction scores. Meanwhile, the AMMI2 biplot reveals genotype-environment interaction effects, aiding genotype stability assessment. Genotypes are considered specific to an environment when showing strong adaptability to it. This specificity is evident in the plot of PC1 scores against genotype and environment mean values. CSJ515 excels in E1 adaptation, while GNG2144 and GNG1958 excel in E2 and RSG974 is specific to E3 (Fig 1B).

The AMMI2 biplot further demonstrates genotype-environment interaction, with genotypes near (0, 0) and within the confidence interval ellipse indicating broad adaptation. GNG2144, GNG1958 and RSG974 are widely adopted. Congruent PC values between genotypes and environments suggest a positive interaction, while incongruent values imply a negative one. Genotypes at distinct coordinates indicate specificity to certain environments; for example, CSG515 excels solely in E1. Synthesizing insights from AMMI1, AMMI2 and genotypic yield data reveals CSJ515's specific adaptation to E1 (Fig 1C).

Genotype + genotype by environment (GGE)

The GGE biplot is a valuable tool for assessing mega-environments, rooted in genotype (G) primary effects and genotype-environment interactions (G×E) (Yan and Rajcan 2002; Yan and Kang 2003; Yan and Tinker 2005). This technique extracts PC1 and PC2 from multi-location experiment data via singular value decomposition (SVD), yielding a two-dimensional representation. Compared to the AMMI biplot, GGE surpasses in mega-environment analysis and genotype assessment. It better reveals genotypic main effects, G + GE interactions and has an

Table 2: Non-parametric stability analysis for seed yield.

Genotype	YSi	Si$^{(1)}$	Zi$^{(1)}$	Si$^{(2)}$	Zi$^{(2)}$	Si$^{(3)}$	Si$^{(6)}$	TOP	NPi$^{(1)}$	NPi$^{(2)}$	NPi$^{(3)}$	NPi$^{(4)}$
CSJ-515	9+	2.00	0.004	3.00	0.002	0.00	0.00	3	1.00	1.00	1.41	2.00
GNG-1958	1	2.66	0.63	4.33	0.40	0.50	0.50	1	1.33	0.33	0.42	0.66
GNG-2144	5+	2.00	0.004	2.33	0.07	0.66	0.66	2	1.00	0.33	0.41	0.66
GNG-2171	2	3.33	2.35	6.33	2.37	1.00	1.00	2	1.66	0.83	0.77	1.25
RSG-959	-2	2.66	0.63	4.00	0.24	0.00	0.00	0	1.33	0.22	0.27	0.44
RSG-974	2	2.66	0.63	4.33	0.40	0.61	0.61	1	1.33	0.26	0.39	0.61

Where,

YSi: Kang's yield and stability index. '+': selected genotypes having YSi> mean of 3.25; Si(1), Si($^{2),}$ Si($^{3);}$ Si($^{6):}$ Nas^{sar} and Huehn's nonparametric stability parameters; SumZi(1): 4.2$^{5;}$ SumZi(2): 3.4$^{9;}$ Chi-sqtabelZi(1); Zi($^{2):}$ 0.3$^{5;}$ Chi-sqtabelSumZi(1), Sum$^{Zi(}$2): 2.1$^{7;}$ TOP: Fox's TOP- Number of sites at which the genotype occurred in the top third of the ranks; NPi(1), NPi$^{(2)}$, NPi$^{(3)}$, NPi$^{(4)}$: The^{nna}rasu's nonparametric stability parameters.

inner product property. GGE introduces discriminating power versus representativeness, useful in evaluating testing environments, not found in AMMI (Yan *et al*., 2007). Its applicability extends to various domains, including mega-environment analysis, genotype evaluation, test environment assessment and heterosis pattern investigation (Yan *et al*., 2002). These qualities make the GGE biplot a popular and comprehensive tool in quantitative genetics and plant breeding.

Utilizing polygons in a GGE biplot is an effective way to visually analyze data. This method serves a dual purpose: identifying optimal genotypes across different environments and clustering environments based on genotype performance, as demonstrated by Yan and Kang (2003). To determine disparities among mega-environments, two key criteria are considered. First, differences in genotype rankings across diverse environments must be evident. Second, variations between clusters should exceed variations within each cluster. The polygon is constructed from the farthest genotype point relative to the axis (0, 0), encompassing all genotypes. Perpendicular lines extend from (0, 0) to each polygon side, dividing the space into sectors with distinct genotype angles. The genotype at each sector's vertex is recognized as the optimal genotype (Yan and Kang, 2003).

The GGE biplot analysis identified five distinct sectors representing different mega-environments, with specific genotypes positioned at the vertices of a polygon. Notably, CSJ515 thrived in sector IV, encompassing environments E1 and E2, while GNG2171 excelled in sector V, linked to environment E3 (Fig 1D). Genotype × environment (GE) interaction is crucial in plant breeding, impacting traits like yield. It complicates predicting genotype performance in target environments based on tests. Careful selection of test locations is vital to account for GE and maximize selection gains. Various statistical methods evaluate GE interaction, including univariate models like regressing genotype means on environmental indices and multivariate approaches. The AMMI and GGE models, along with biplot visualization, are popular for analyzing multi-environment trial data. Although proponents of these methods may disagree on their superiority, they generally yield similar results (Gauch, 2006; Yan *et al*., 2007).

Stability analysis in agricultural research plays a pivotal role in evaluating genotype performance across diverse environments. Parametric measures, reliant on statistical assumptions, can be influenced by outliers in small samples. Alternatively, nonparametric rank-based stability measures offer robustness without distribution assumptions and are less sensitive to measurement errors. This approach, comparing genotype ranks across environments, identifies stable performers. The study's findings illuminate the adaptive stability of various genotypes. Among them, CSJ515 consistently displayed remarkable stability across all 10 analyses, making it a dependable choice for cultivation in diverse conditions.

Table 3: Spearman correlation between stability parameters.

	Yi	CVi	bi	s^{2}di	Wi^2	Di	σ^2	YSi	Si$^{(1)}$	Si$^{(2)}$	Si$^{(3)}$	Si$^{(6)}$	TOP	NPi$^{(1)}$	NPi$^{(2)}$	NPi$^{(3)}$
Yi																
CVi	-0.31															
bi	-0.20	-0.37														
s^{2}di	0.03	0.37	0.14													
Wi^2	0.03	0.37	0.14	1.00**												
Di	0.03	0.37	0.14	1.00**	1.00**											
ó^2	0.03	0.37	0.14	1.00**	1.00**	1.00**										
YSi	0.90*	-0.06	-0.17	0.23	0.23	0.23	0.23									
Si$^{(1)}$	0.28	0.31	0.09	0.77	0.77	0.77	0.77	0.63								
Si$^{(2)}$	0.12	0.32	0.38	0.81*	0.81*	0.81*	0.81*	0.46	0.94**							
Si$^{(3)}$	-0.29	0.52	-0.23	0.81*	0.81*	0.81*	0.81*	-0.18	0.39	0.38						
Si$^{(6)}$	-0.29	0.52	-0.23	0.81*	0.81*	0.81*	0.81*	-0.18	0.39	0.38	1.00**					
TOP	0.97**	-0.38	-0.09	0.15	0.15	0.15	0.15	0.93**	0.43	0.27	-0.24	-0.24				
NPi$^{(1)}$	0.28	0.31	0.09	0.77	0.77	0.77	0.77	0.63	1.00**	0.94**	0.39	0.39	0.43			
NPi$^{(2)}$	-0.90*	0.58	0.17	-0.06	-0.06	-0.06	-0.06	-0.74	-0.20	-0.01	0.18	0.18	-0.93**	-0.20		
NPi$^{(3)}$	-0.83*	0.60	0.26	-0.03	-0.03	-0.03	-0.03	-0.64	-0.12	0.09	0.12	0.12	-0.85*	-0.12	0.99**	
NPi$^{(4)}$	-0.90*	0.58	0.17	-0.06	-0.06	-0.06	-0.06	-0.74	-0.20	-0.01	0.18	0.18	-0.93**	-0.20	1.00**	0.99**

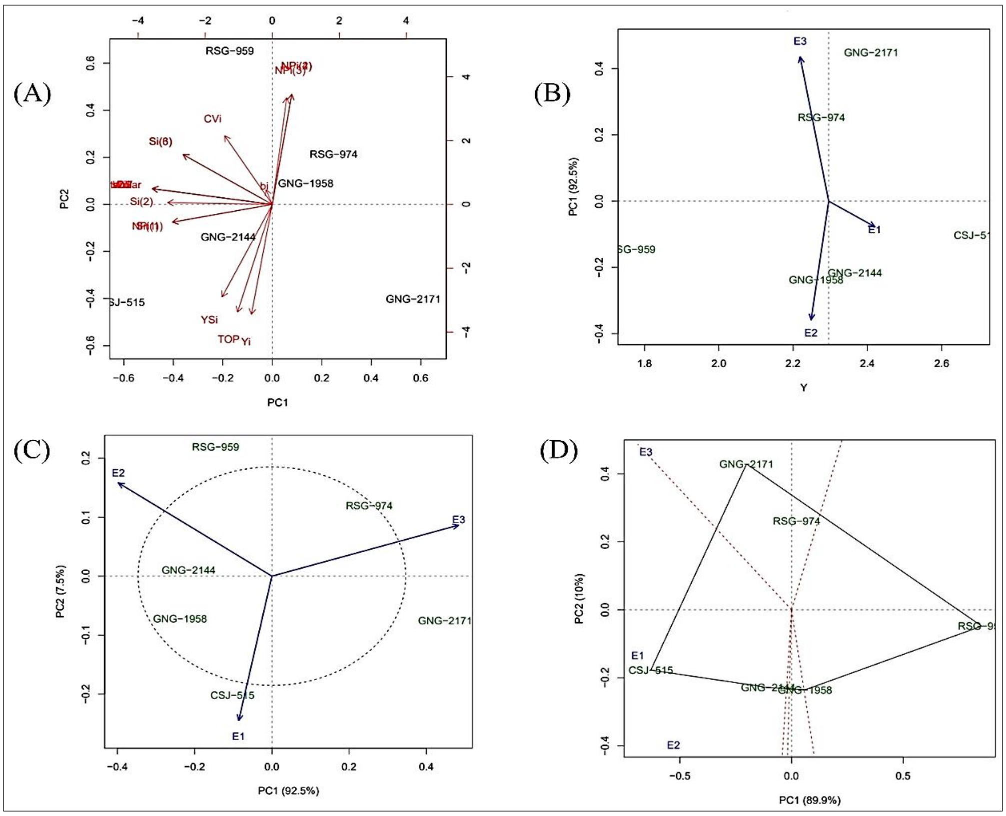

Fig 1: (A) PCA biplot of genotype and stability parameters (B) Biplot AMMI-1 (C) Biplot AMMI-2 (D) GGE biplot with mega-environment sector.

GNG2144 and RSG959 also exhibited notable stability, excelling in 7 out of 10 analyses. While not as universally stable as CSJ515, these genotypes showcase strong adaptability and hold promise for widespread cultivation. These insights are essential for sustainable agriculture, guiding genotype selection to minimize yield fluctuations in ever-changing environments (Fox *et al*., 1990; Huehn, 1979).

GNG1958 displayed moderate stability in four out of ten analyses, suggesting potential for specific environmental adaptation. Similarly, GNG2171 and RSG974 showed stability in 3 and 2 analyses, indicating their suitability under certain conditions. CSJ515, GNG2144 and RSG959 exhibited robust and consistent stability. These findings are significant for crop selection and breeding strategies, offering insights into sustainable crop production. Further research can explore the genetic and physiological mechanisms behind this stability.

CONCLUSION

The genotype CSJ515 exhibited consistent stability across 10 stability analyses. Two genotypes, GNG2144 and RSG959, consistently demonstrated stability in 7 stability analyses. GNG1958 displayed consistent stability in four stability analyses, while GNG2171 and RSG974 exhibited consistent stability in 3 and 2 stability analyses, respectively. Overall, it can be inferred that the genotypes CSJ515, GNG2144 and RSG959 displayed broad and stable adaptation.

Conflict of interest

The authors disclose that there is no potential conflict of interest.

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