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GGE Plot Analysis for Identification of Location Specific Stable Genotypes and Suitable Environment for Chickpea root Nodulation

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ABSTRACT: The ability of chickpea to obtain sufficient nitrogen via its symbiotic relationship with *Mesorhizobium ciceri* is of critical importance in supporting growth and grain production. A number of factors can affect this symbiotic relationship including abiotic conditions, plant genotype, soil and environmental conditions. Hence, the present study was aimed at facilitating the GGE biplot analysis through multi-environment testing of chickpea genotypes, in order to select the diverse and stable nodulating genotypes across the locations, we have evaluated fifty-seven genotypes in four different locations during rabi 2021-22. Based on the results of hierarchical clustering the genotypes are divided mainly into two clusters with three sub clusters in cluster 1 and three sub clusters in cluster II indicating presence of considerable variability or dissimilarity among the evaluated genotypes. Based on the principal component analysis, the first 2 PCs accounted for 76.4% of the total variation. The PC1 explained 49.4 % of total variation, while PC2 explained 26.6% total variability. The genotypes IC 11307, ICC3734, IC 814 constituting PC1 and ICC13153, ICC8143, ICC1770 constituting PC2 contributed maximum genetic variability particularly through the trait nodule fresh weight. Thus, we could identify the genotypes, *viz.*, ICC 16409, ICC 6120, ICC 14916, ICC 5434 and ICC 5146, which were found to be highly stable with high mean yield and these genotypes would serve as donors for further improvement of nodulation. Further, the environment E1 (IARI-New Delhi) was identified as most suitable representative for nodulation and high yield. Thus, identified stable genotypes can be used in breeding for developing verities with high biological nitrogen fixation.

Keywords: GGE biplot, Hierarchical clustering, Nodulation, PCA, stability.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the third most important globally cultivated legume. India is the leading producer of chickpeas with an annual production of 11.5 million tons (FAOSTAT, 2023). The chickpea like other legumes has the capability to convert atmospheric nitrogen (N2) into ammonia (NH3) in symbiotic association with *Mesorhizobium ciceri and it* promote growth and facilitating grain yield. Farmers exploit this mutually beneficial interaction with rhizobia to overcome nutrient deficiencies in soils, as these bacteria can supply as much as 97% of a plant's total nitrogen demand Peoples and Craswell (1992). In addition, these symbiotic relationships play a crucial role in replenishing substantial amounts of nitrogen in agricultural soils and thereby decreasing the reliance on expensive fertilizer treatments worldwide Herridge *et al*., 2008). The success of breeding programs is highly dependent on the availability of donor genotypes having consistent and stable performance over multi-year and multi-location environments (Parihar *et al*., 2017) and existence of genetic variability in a particular crop species (Manraranjan *et al*., 2021; Goutham *et al.,* 2021). This is because quantitative traits like root nodulation in a plant are highly influenced by the current environment relative to spatio-temporal climatic variations. The terms 'stability' or 'adaptability' refer to consistent high performance of genotypes across diverse sets of environments. In order to identify the most stable and high yielding genotypes, it is important to conduct multi-environment trials (Lu'quez *et al*., 2002). Genotypes tested in different environments often have significant fluctuations in yield due to the response of genotypes to environmental factors (Kang, 1993). These fluctuations are often referred as genotype × environment interaction (GEI). A more recent method, the graphical GGE {genotypic main effect (G)

plus genotype and environment interaction (GE)} biplot technique, is now being used widely to evaluate the stability of genotypes, environment, and consequent genotype *vs* environment (G × E) interaction obtained from multi-environment trials (Parihar *et al.,* 2017**).** Thus, evaluating the individual and combined impacts of the genotypes and environment on root nodulation will play a key role in identifying superior and stable chickpea genotypes. The utilization of available germplasm for root nodulation traits may be enhanced by characterizing them through extensive phenotyping. In this regard, we have carried out our research on identifying better nodulation genotypes across the varied tested environments.

MATERIALS AND METHOD

Experimental conduct and phenotyping for Number of nodules. We have evaluated fifty-seven (Supplementary Table 1) genotypes chosen from an association panel for number of nodules extracted from core germplasm obtained from ICRISAT, Patencheru, Telangana, India (Upadhyaya, 2003). Fifty-seven genotypes were selected on the basis of their high mean value for number of nodules and nodule fresh weight. Multilocation experiments were set up in a randomized complete block design (RCBD) in the year 2021-2022 during rabi seasons at IARI, New Delhi{Environment 1(E-1)}(Fig. 1).; SHUATS, Allahabad {Environment 2(E-2)}; RPCAU, Samastipur, KVK Vaishali campus{Environment 3(E-3)}; and IARI regional station, Pusa, Bihar{Environment 4(E-4)}. The increased spacings of 60 cm between row to row and 10 cm between plant to plant were maintained. The following observations were taken namely; Number of nodules per plant (NON/plant), Nodule fresh weight (NFW) in milli gram (mg), Number of pods per plant (NPP), Number of seeds per plant (NOS/plant), Seed yield (SY) in gram (g) per plant, 100 Seed weight (100SW) in g. The phenotyping was done at 60 DAS stage when plants were in 50% flowering with complete vegetative growth. No external inoculation of *Rhizobium* was done to get a natural variation in nodulation. Five randomly selected plants from each genotype were uprooted with the adhered soil mass using a hand hoe by digging 20 cm or even deeper into the soil and the adhered soil was removed carefully. Root and shoot systems were separated. Roots with intact nodules were washed and counted for number of nodules and stored in butter paper bags for further taking nodule fresh weigh.

Statistical analysis. Construction of the Genotype plus Genotype × Environment Interaction biplot were accomplished. The genotype (G) and genotype by environment (GE) interaction were graphically represented by the GGE biplot based on the scores of first two principal components (PC) resulting from the singular value decomposition (Yan *et al.,* 2000; Yan, 2002; Yan and Hunt 2001; Yan and Kang 2003).

$\mu Yij = \mu + ej + \sum n=1 N \lambda n \gamma in \delta jn + \varepsilon ij$

Where, Y_{ij} = mean response of the ith genotype (i = 1,…,i) in the j^{th} environment (j = 1,., j); μ = grand mean; e_j = environment deviations from grand mean; λ_n = eigenvalue of PC analysis axis; γ_{in} and δ_{jn} = genotype and environment PC scores for axis n; N = number of PCs retained in model; ε_{ij} = residual effect ~N (0, σ^2e).

Data analysis and software. The cluster heat map was performed using the "heatmap.2" function through the plots v3.0.1.1 library implemented in R.A popular method of dimension reduction is PCA (Massey, 1965; Jolliffe, 2002) was performed in R software. The GGE biplot, GT biplot, and GYT biplot were created utilizing GGE biplot package in R software.

Fig. 1. Field view of experiment plot of an association panel.

RESULTS AND DISCUSSIONS

A. Evaluation of genotypes for genetic variability and diversity

The genotypes were evaluated for nodulation and yield contributing traits under above mentioned locations and the results are presented as follows. Pooled analysis of variance has shown significant differences among the tested genotypes. The results of the Hierarchical clustering (Fig. 2) showed that 57 genotypes are divided mainly into two clusters. The cluster I was divided into three subclusters and cluster II into three sub clusters. Thus, the presence of 6 subclusters indicate enough variability or dissimilarity among the evaluated genotypes. Narrow genetic variation had been reported in chickpea germplasm by various researchers (Yadav *et al,* 2003; Singh *et al.,* 2007; Upadhyaya *et al.,* 2008). Studies on genetic diversity and relationships among landraces and improved varieties are not only useful for germplasm conservation, but also facilitate use of the genetic resources in crop improvement programmes (Chandana *et al*., 2023; Choudhary *et al.,* 2012; Kumar *et al.,* 2017).

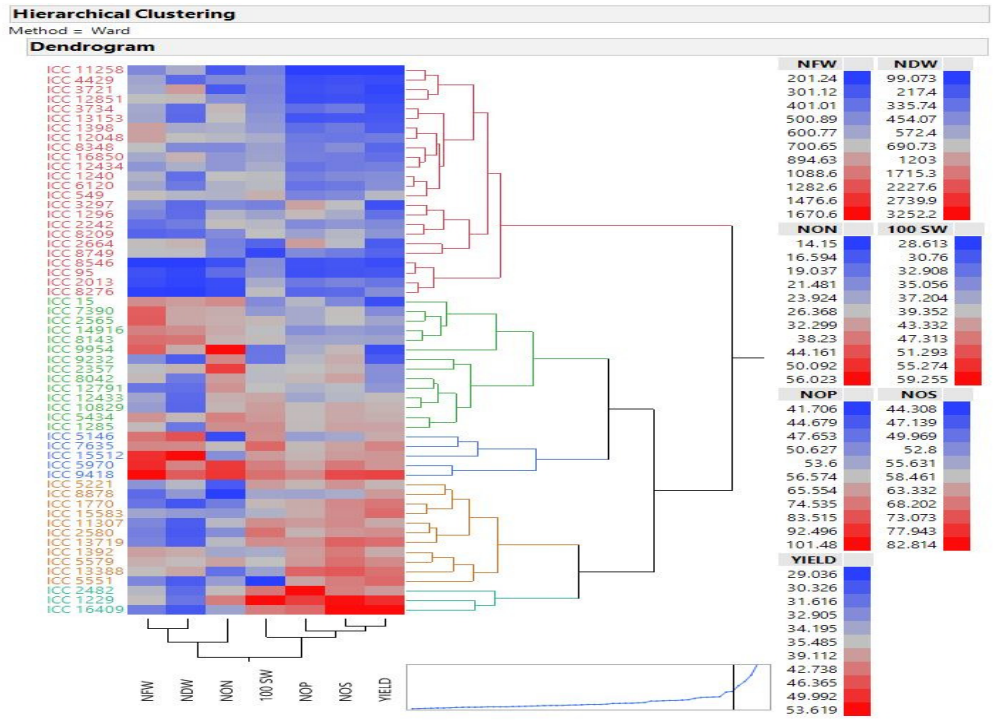
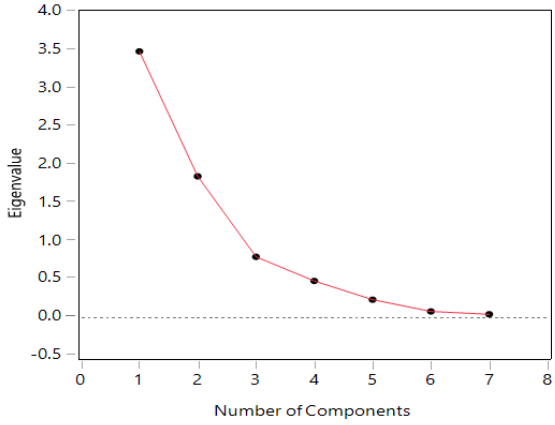


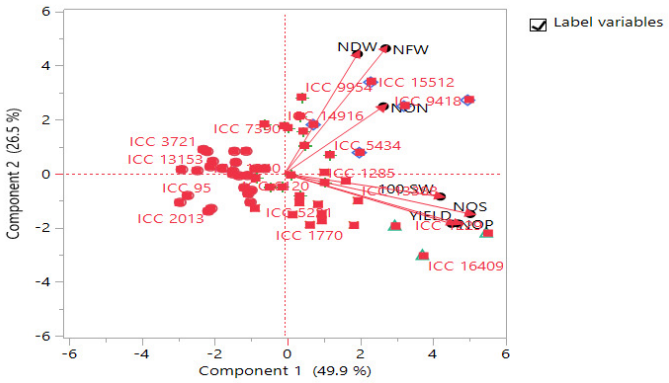
Fig. 2. Hierarchical clustering of genotypes.

B. Principal component analysis

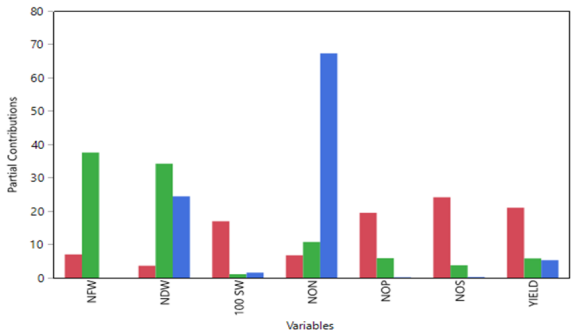
The selected genotypes were used for estimating principal component analysis along with screen plot in order to define the criteria for selection of genotypes and traits. Screen plot explained the percentage of variation associated with each principal component obtained by drawing a graph between Eigen value and principal component number (Fig. 3a). Principal component (PC)1 had explained 49.99% variability followed by 26.5%, 11.42%, 6.9%, 3.4% and 1.2% respectively by PC2, PC3, PC4, PC5 and PC6 (Fig. 3b and 3c). PC1 which accounted for the highest variability was contributed by NFW, test weight and yield. PC 2 was dominated by NFW, NOP, NON and yield. Similar contributions have also been reported by NOP earlier (Rekha *et al.,* 2014). The maximum PC values were contributed by the genotypes IC 11307, ICC3734 followed by IC 814 and ICC 95 for PC1. The genotypes ICC13153, ICC8143, ICC1770 have contributed maximum variability for PC2. The genotypes found common for both PC1 and PC2 were ICC 95, ICC 7635, and ICC 1552. The intensive selection procedure can be designed to bring out rapid improvement of dependent traits *i.e.,* NON and NFW by selecting the lines of PC 1, PC2 and PC3 as suggested earlier that a high value of PC scores can be used for selection of potential genotypes for their further utilization in future breeding programmes (Iqbal *et al*., 2008; Amrita *et al*., 2019).



(a)



(b)



(c)

Fig. 3: **(a)** Screen plot based on PCA and eigen values (**b)** Distribution of traits of chickpea based on principal component PC1 and PC2 (**c)** Total variance explained by different traits to principal components.

The genotypes which are common in more than 1 PCs indicate that selection of genotypes from these PCs will be useful in further crop improvement programmes. Thus, genetic diversity analysis from hierarchical clustering and PCA facilitate use of these germplasm lines for selecting diverse lines for nodulation or biological nitrogen fixation.

GGE biplot analysis. Efficiency of genetic gain received through selection is seriously affected by genotype × environment (G × E) interaction, as G×E directly affects the stability and performance of genotype under multi environment. The current study was conducted to gain insights into the G × E effect and the stability of genotypes grown across the sites. Yield data recorded from the genotypes were analysed by using GGE bi-plot method. The effects of genotype, environment, and genotype × environment was found to be significant (Table 1).

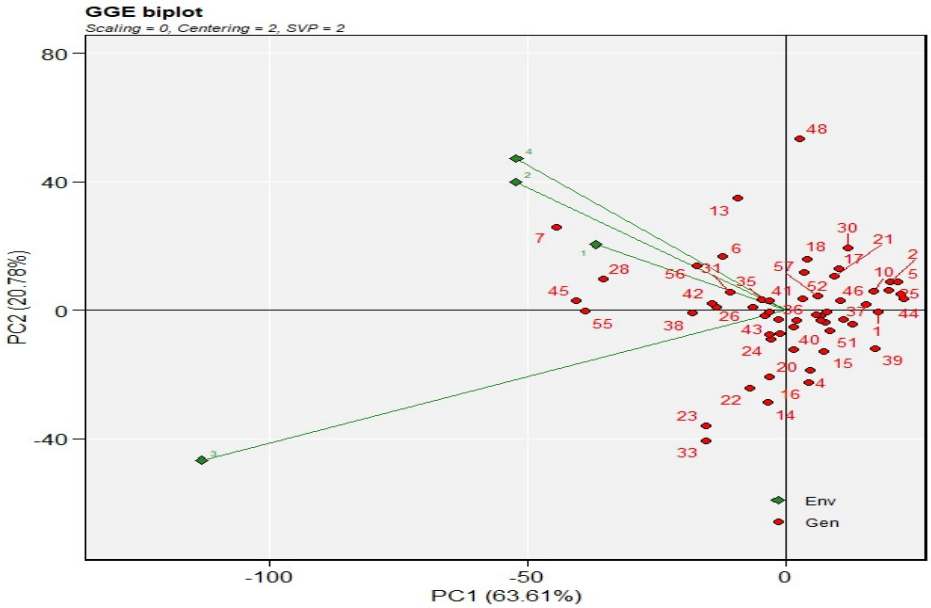


Fig. 4. GGE biplot analysis for genotype and environment interaction.

Table 1: Analysis of variance for fifty seven genotypes of chickpea evaluated under four different environments.

Sources of variation	DF	Sum of squares	Mean sum of squares	F value	Pr>F
Genotypes	56	434.28	17.65	24.4	0.00
Environments	3	183.45	21.53	29.8	0.00
G× E	168	745.34	4.98	5.4	0.00
Residual	224	184.36	0.8	-	-
CV	15.67				

The results of GGE biplot analysis further elucidated the relative contribution of first two PCs axes to the interaction effects by plotting with genotype and environment means (Fig. 4) environments are designated by green colour and number 1 to 4 as suffix. The genotypes are designated by red colour and suffixed by the numerals as 1.2,3…...57. When a genotype and the environment have similar sign on PCA1 axis their interaction is meant positive and if opposite, their interaction is considered negative. Therefore, a variety with near to zero PC1 score means that it had small interaction effect and hence was considered as stable over wide environments. In our study the genotypes 26(ICC 8042), 31(ICC 12791), 36(ICC 10829), 38(ICC 5434), 44(ICC 8878), and 56 (ICC 16409) with high mean yield and large PCA scores were regarded as clearly adapted to specific environments as supported by several others (Abdi and Williams, 2010; Askari *et al.,* 2017; Mustapha and Bakari 2014). Accordingly, in the present study, the chickpea genotypes ICC 3721 and ICC 4429 (genotypes 2 and 4), being the overall best performer are exhibiting high yield with positive IPCA 1 score among all genotypes at location1. On the other hand, the genotype ICC5579 was best for location 2; ICC 7390, ICC 2565 (genotypes 23,33) for location 3, and ICC 1398 for location 4. So, the genotypes placed near the origin were regarded as stable one compared to others. Likewise, genotypes lying away from the origin and having long spokes were considered as highly interacting types. Environments E1, E2 and E4excreted less interaction forces, while, E3had strong interaction effects.

Mean *vs* stability. Ranking of 57 genotypes based on mean yield and stability (Fig. 5). The line passing the biplot origin from upper right to lower left is known as average environment axis (AEA). The line passing through the origin and perpendicular to AEA with double arrow denotes stability of genotype (Yan and Hunt 2002). The genotypes existing in either direction away from the origin on the axis indicate G × E interaction and low stability (Yan and Hunt 2002). The genotypes 57(ICC 16409), 27(ICC 6120, 29 (ICC 14916), 38(ICC 5434) and 39(ICC 5146) were found to be highly stable with high mean yield.

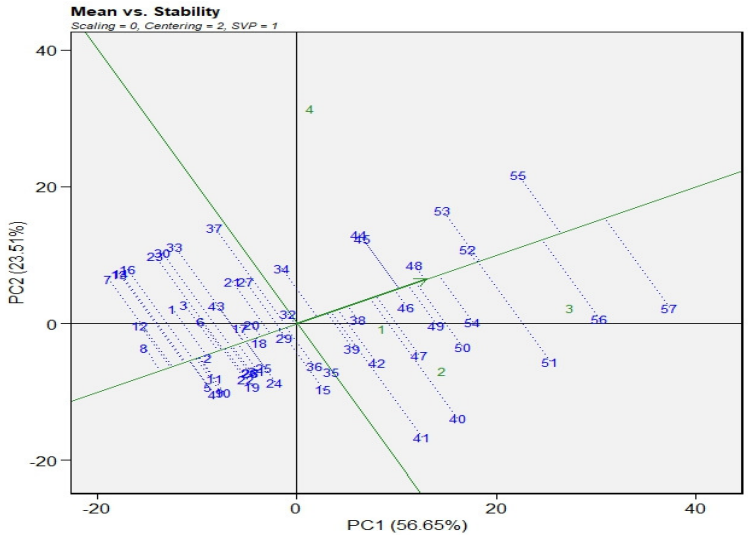


Fig. 5. Mean *vs* stability pattern of GGE biplot illustrating interaction effect.

Which won where polygon. A "which won where" polygon view of the relationship between genotypes and environments (Fig. 6). Genotypes appeared on the vertices of the polygon suggest the best or the poorest in one or another environment (Yan and Hunt 2002). The genotypes appeared on the corners of "which won where" polygon are revealing that they were the best genotypes in specific environments accordingly, the genotypes such as 20(ICC 12048), 32(ICC 8143), 36(ICC 10829) and 43(ICC 2482), are considered as stable across all the evaluated environments. However, the genotypes 51(ICC 13388), 56(ICC 1229) and 57(ICC 16409) were found be highly adapted to adapted location 1 and 2.

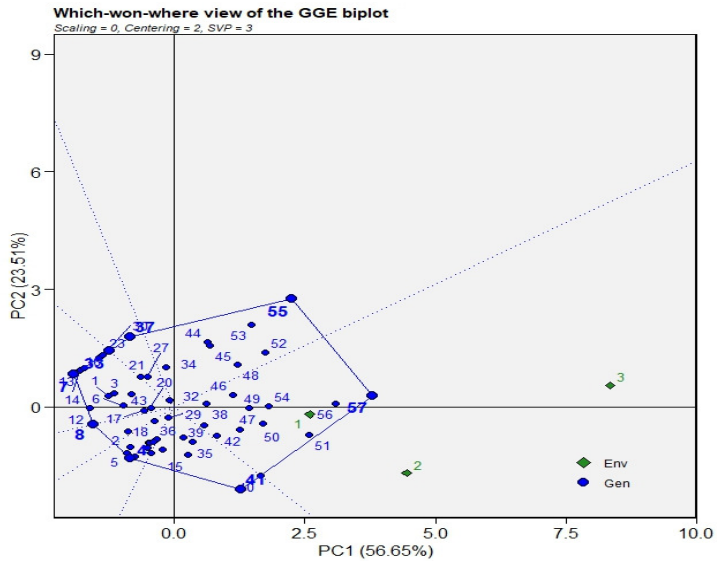


Fig. 6. 3 GGE biplot for identification of winning cultivars across four environments.

GGE Biplot on environment for comparing environmental efficiency. Te determination of a best suited (ideal) test environment is crucial for a successful breeding technique in the selection of superior genotype An ideal environment represents the highest discriminating power. (Yan and Tinker 2006) and evaluation of test environment could help in identifying environments that could be utilized for selecting superior genotypes for mega environment (Rashidi *et al.* 2013; Sousa *et al*., 2018). Differentiating capability and precise representation of testing environments are two pivotal roles of GGE biplot. The concentric circles d (Fig. 7) aid in visualizing the length of environment vectors, which are the indicators of the discriminating ability and standard deviation within the respective environments (Kang-BoShim *et al.,* 2015). Thus, the environment E3 considered as most discriminating, the environment E1 as the most representative, whereas the environments E2 and E4 are the least representative. Test environments possessing discriminating ability as well as representativeness are highly suitable for selecting adaptable genotypes. Discriminating but non representative test environment like E2 and E4 are useful for selecting adaptable genotypes.

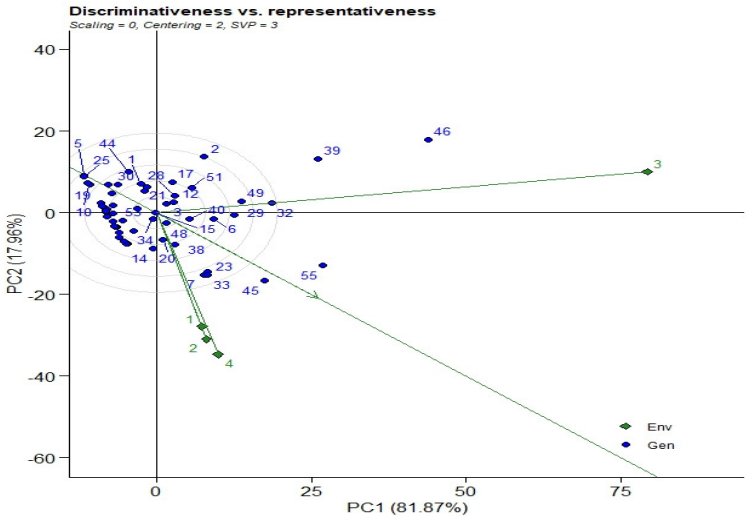


Fig. 7. GGE biplot based on environment-focused scaling for comparison of the environments with the ideal environment.

Ranking genotypes relative to the ideal genotypes. Genotypes relative to an ideal genotype is depicted in (Fig. 8). The arrow depicts the position where an ideal cultivar should be grown (Frutos *et al.,* 2014). In the genotype focused GGE biplot analysis, concentric circles are drawn to help visualize the distance between each genotype and the ideal genotype (Naroui Rad *et al.,* 2013). An ideal genotype is located in the first concentric circle of GGE biplot graphic and the genotypes that are close to the ideal genotype are defined as the desired genotypes. The genotypes 50(ICC 13388), 51 (ICC 13719), 52 (ICC 15583), 53 (ICC 5551), 54 (ICC 9418), 55 (ICC 1229), and 56 (ICC 16409) may be considered as desirable genotypes in relation to the ideal genotype.

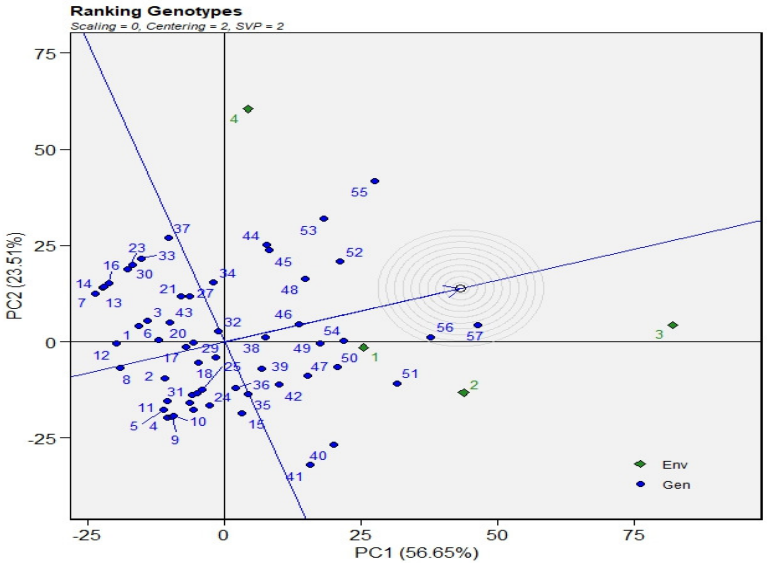


Fig. 8. GGE biplot based on genotype – focused scaling for comparison.

CONCLUSIONS

The present study showed the importance of undertaking multi-location trials, their subsequent G × E interactions, and stability analysis for high nodulation and their positive response on biological nitrogen fixation and increasing yield. The results of hierarchical clustering indicated the presence of sufficient genetic diversity among the evaluated genotypes that may be useful for future breeding program. The principal component analysis found more than 70% of variation from two components of PCA. PC1 accounted for the highest variability was contributed by NFW and PC2 by was dominated by NFW, NOP, NON and yield. Thus, PCA analysis has suggested that the genotypes IC 11307, ICC3734, IC 814 constituting PC1 and ICC13153, ICC8143, ICC1770 constituting PC2 would be of practical value to chickpea breeders in identifying the genotype with desired trait(specifically for nodule fresh weight and high number of nodules for utilization in breeding. GGE biplot analysis permitted estimation of interaction effects of genotypes in each environment that helped to identify genotypes best suited for specific environments. The genotypes ICC 16409, ICC 6120, ICC 14916, ICC 5434 and ICC 5146were found to be highly stable with high mean yield and the environment E1(IARI, New Delhi) was identified as most suitable representative for nodulation and high yielding stable genotypes.

FUTURE SCOPE

Our findings have broadened the scope for presence of diverse root nodulation genotypes that can serve as donors or parents in breeding programme for developing high root nodulation genotypes and thereby improving the biological nitrogen fixation and achieve soil sustainability. The genotypes with high value of PC scores, which are common in more than 1 PCs can be used for selection and their further utilization in future breeding programme. Further, if agronomically superior, the identified stable genotypes could be cultivated at large scale for higher chickpea productivity.

Author contributions. Conceptualization, designing and monitoring of research by RK, Execution of experiments and data collections by CBS, RKM, RKS, KKS, SK and GRL. The data analysis and interpretations by CBS and DS and RK, authors edited and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest. None.

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