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Identification of stable drought tolerant landraces of chickpea (*Cicer arietinum*) under multiple environments

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ABSTRACT

Drought is a major constraint to chickpea production leading to maximum crop loss. Further the narrow genetic base of chickpea (*Cicer arietinum* L.) can be widened by crossing them to landraces and wild species of which landraces provide valuable sources for abiotic and biotic stresses. The present investigation identifies highly stable drought tolerant landraces using AMMI analysis and GGE biplot techniques. The 42 chickpea genotypes (38 chickpea landraces obtained from West Asia and North Africa (WANA) and 4 known varieties) were evaluated at two locations under randomized block design in irrigated and rainfed condition in two seasons. Additive main effects and multiplicative interaction (AMMI) and Genotype main effect and genotype × environment interaction (GGE) were employed in the evaluation of genotype. AMMI analyses decomposes SS for GEI in to 3 Interaction Principal Components(PC) of which PC1(79.6%) and PC2(17.8%) explains most of the variability. From AMMI Stability Value (ASV) the genotype G35 (IL184) and G23 (IG5895) were found most stable landraces while Yield Stability Value (YSI) ranking identified G8 (IG5856) as the best genotype, based on stability and mean yield. From GGE biplot analyses the PC1 explains 80.0% and PC2 explains 18.9% of variability. G8(IG5856) performs well under across all the environments with high mean yield. Drought Susceptible Index (DSI) indicated G8 (IG5856) and G2 (ICC4958) to have the lowest DSI at both the locations. From the above investigation the landrace IG 5856 from Jordan was found to be most drought tolerant.

Key words:AMMI, Chickpea, Drought, GGE, Landraces

Chickpea (*Cicer arietinum* L.) is the important food legume, in Indian subcontinent grown in winter season with receding soil moisture condition after rainy season. The drought is the main abiotic constraint which account for 50% yield loss in chickpea (Varshney *et al*. 2010). With climate change and population explosion there is urgent need to develop drought tolerant high yielding chickpea varieties (Krishnamurthy *et al*. 2013a). Chickpea grown in Indian subcontinent has a narrow genetic base (Bharadwaj *et al*. 2011) which is limiting the crop improvement through conventional efforts. Chickpea landraces have a broad genetic base and vast genetic diversity and they can be utilized for base broadening and gene introgression for development of drought tolerant variety. Evaluation of landraces for yield under drought condition and calculation of Drought susceptibility index by Fischer and Maurer (1978) helps in identification of lines having drought tolerance.

The differential behaviour of genotypes under the influence of GEI will bias the genotype selection. Multiple environmental trials (MET) were used to study adaptability and stability of genotype and identification of wide and specifically adoptable genotypes. Interpretation of performance of genotypes in a broad range of environments is generally affected by GEI (Gauch and Zobel 1996). For evaluating GEI various statistical techniques were used of which AMMI and GGE Biplot were most frequent. AMMI model proposed by Gauch (1992) uses analysis of variance and principal component analysis to achieve a better understanding of GEI, its causes and consequences. Yan *et al*. (2000) proposed the GGE Biplot analysis, which considers both genotype main effects and GEI effects as important for the analysis. Present study was conducted on 42 chickpea genotypes which includes 38 landraces and four known varieties evaluated under irrigated and rainfed condition at two locations IARI and Dharwad.

MATERIALS AND METHODS

Experimental materials and experimental design

The 42 chickpea genotypes (Table 3) evaluated comprised of 38 landraces collected from WANA region and 4 check varieties. Experiment was conducted at IARI (28.080^{0}N and 77.120^{0}E) and Dharwad (15.4589^{0}N and 75.0078^{0}E) in two seasons 2017-18 and 2018-19 under

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irrigated and rainfed conditions (Table 1). Experiment was conducted in randomized block design. Average yield per plant in gram was taken for analyses.

Drought Susceptibility Index (DSI)

Drought susceptibility index was estimated as per Fischer and Maurer (1978).

$$DSI = (1\text{-}Y_S/Y_N) \div (1\text{-}y_S/y_N)$$

where, Y_S and Y_N = mean yield of individual genotype under rainfed condition and irrigated condition, y_S and y_N= mean yield of all genotypes under rainfed condition and irrigated condition.

AMMI statistics

AMMI analyses were done after performing ANOVA which provided a preliminary indication as to whether AMMI analysis will be worthwhile. Sum of square (SS) for G (Genotype) and GEI (Genotype × Environmental Interaction) are direct outcome of ANOVA (Gauch 1992). The product of error mean square and number of degrees of freedom (df) for GEI will give the GEI noise (GE_N) and GEI signal (GE_S) were calculated by subtracting $GE_N$from GEI. AMMI analysis is appropriate for datasets that have substantial G and substantial GE_S. Especially when the SS for GE_S is at least as large as that for G, as happens frequently, AMMI analysis will probably be worthwhile (Gauch 2013).

The AMMI model equation is written as: $Yge = \mu + \alpha_g + \beta_e + \Sigma_n\lambda_n\gamma_{gn}\delta_{en} + \rho_{ge}$. Where, Yge = yield for genotype (g) in environment (e), μ = grand mean, α_g= genotype deviation, β_e= environment deviation, λ_n = singular value for component n, γ_{gn}andδ_{en}= eigenvector value for g and e and ρ_{ge}= residual (Gauch and Zobel 1997). The G×E interaction was analysed in an AMMI model (Zobel 1988, Guach 1992).

AMMI Stability Value (ASV) and Yield Stability Index (YSI)

AMMI's stability value (ASV) was proposed by Purchase (1997) were calculated as by using follows:

AMMI stability value (ASV)

$$\sqrt{\left[\frac{IPCA1\ sum\ of\ square}{IPCA2\ sum\ of\ square}\left(IPCA1_{score}\right)\right]^2 + \left(IPCA2_{score}\right)^2}$$

where, $IPCA1_{\text{sum of squares}}/IPCA2_{\text{sum of squares}}$ are the weight given to IPCA1 and IPCA2. The larger the IPCA score, either negative orpositive, the more specifically adapted a genotype is to certain environments. Smaller ASV scores indicate a more stable genotype across environments. Theyield stability index (YSI) was calculated as:

$$YSI = RASV + RY$$

where, RASV is the rank of the AMMI stability value and RY is the rank of the mean grain yield of genotypes (RY) across environments.

Table 1 Environment used for screening chickpea landraces

Codes	Environments
E1	Dharwad Irrigated
E2	DharwadRainfed
E3	IARI Irrigated
E4	IARI Rainfed

GGE Biplot analyses

The GGE biplots were drawn as described by Yan and Kang (2003). Genotype-focused scaling was used in visualizing for genotypic comparison. The morphological data were analyzed using the software "GEA-R (Genotype × Environmental Analysis with R windows) version 4.1",CIMMYT Research Data and software Repository Network, V16 and PBTools1.4 (IRRI, Philippines).

RESULTS AND DISCUSSION

Drought Susceptible Index (DSI)

The DSI was the most widely used criteria to determine the tolerant genotype (Fischer and Maurer 1978). The results from the analysis shows the landraces G8 (IG5856), G2 (ICC4958), G25(IG5904) and G15 (IG5866) had a low DSI of 0.0255, 0.0648, 0.12621and 0.2836 at IARI and 0.0335, 0.0630, 0.1708 and 0.1728 at Dharwad, respectively indicating that they perform well under both irrigated and rainfed condition and also have wider adaptation, among these G8 had higher mean yield. The genotype IG5856 and IG 5904 were also reported to be drought tolerant by Kumar *et al*. (2015).

AMMI analysis

Combined analysis of variance

The results from Table 2 for ANOVA for grain yield per plant in chickpea shows that the sum of squares (SS) for G is 9823.48, for GE_N is 51.15 and for GE_S is 2411.47 indicating the appropriateness for carrying out AMMI

Table 2 Combined analysis of variance of the productivity of chickpea trials and Decomposition of the sum of squares of (GEI)

Source	Df	SS	MS	F_G
ENV	3	1265.533	421.844	1014.11***
GEN	41	9823.48	239.597	575.99***
ENV*GEN	123	2462.61	20.021	48.13***
PC1	43	1959.142	45.5614	134.86***
PC2	41	439.500	10.7195	31.72***
PC3	39	63.9761	1.64042	4.855**
Residual	168	69.883	0.4159	
Total	335	13621.50	-	-
Grand mean = 12.80				
CV= 5.03 %				

analysis as the ANOVA has retained the substantial level SS for G and GE_S (Gauch 2013). The results provided by the AMMI combined analysis of variance for yield (g per plant) in chickpea landraces showed the presence of significant genotypic and environmental main effects as well as interaction effects (P<0.001).The relative magnitude of SS for genotype, environmental and GEI variance was 72.18, 9.29% and 18.07%. The differential performance of a genotype under the influence of environment was indicated as significant GEI also by Kanouni *et al*. (2015). The high genotype variance among the landraces shows that it is possible to identify drought lines.

GEI was further partitioned by principal component analysis (Table 2). Ordination technique using an approximate F-statistic (Gollob 1968) revealed high significant differences for interaction for PC1, PC2 and PC3. The interaction PC1 (79.6 %) and PC2 (17.8 %) together explained 97.4% of the variability, with significant effect of P<0.001(Gollob 1968). Since 70% is considered the minimum amount of variability for the model to be relatively reliable (Neisse *et al*. 2018), hence the current analysis is significantly reliable.

Biplot analyses

To investigate the main effects and interactions, AMMI1 biplot was constructed for yield (Fig 1). The mean yield or additive main effects are shown along abscissa and first Interaction PC or multiplicative interaction on ordinate of biplot. The results from Fig 1 shows that the landraces G23 (IG5895), G37 (ILC239), G8 (IG5856) and G15 (IG5866) were having low PC1 (score irrespective of their sign) indicating negligible or zero interaction effect. But when we consider mean yield along with PC1 score, the landraces G8 (IG5856) ranks first followed by G25 (IG5904) and G2 (ICC4958) and were stable and high yielding landraces. On biplot those landraces on right side of grand mean are high yielding landraces and those landraces and environment that have same sign have positive interaction. Environment E4 (IARI irrigated) has highest mean yield fallowed by E2 (Dharwad irrigated). AMMI Analysis was also conducted and the stability of genotypes was predicted on the basis of mean performance and the magnitude of IPCA1 score in chickpea (Farshadfar and Mohammad 2013).

AMMI2 Biplot

The AMMI2 biplot in Fig 2 for The PC1 versus PC2 explains the magnitude of interaction of each genotype and environment. The E3 (IARI rainfed) has shorter vector distance so this is most stable environment and the landraces G27(IG5909), G24 (IG5896), G37(ILC239), and G35 (ILC167) which were near to centre of biplot indicating that they were most stable landraces across the irrigated and drought condition. The landraces G18(IG5870), G11(IG5862) and G26(5906) perform specifically well under E2 (Dharwad irrigated) and the landraces G17(IG5868), G16(IG5867) and G5 (IG5844A)

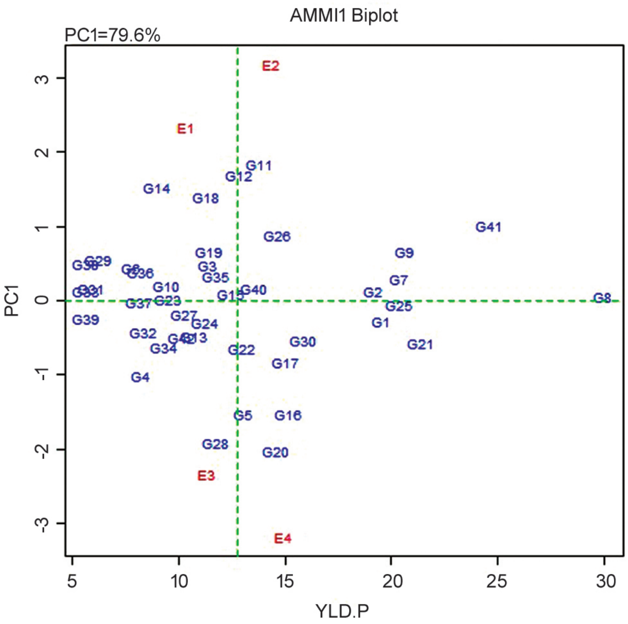

Fig 1 AMMI1 Biplot chickpea mean yield per plant versus interaction principal component1 (PC1).

in E4 (IARI irrigated). Such differential response of genotypes in different environments was also found in chickpea by Farshadfar and Mohammad (2013).The genotypes and environments that are farthest from the origin are more responsive fit the worst. The genotypes will interact positively if genotypes and environments that fall into the same sector, negatively if they fall into opposite sectors (Osiru *et al*. 2009).AMMI2 is also useful for the delineation of mega-environments, that is, group's of environments that have the same genotype as most productive (Hongyu *et al*. 2014).

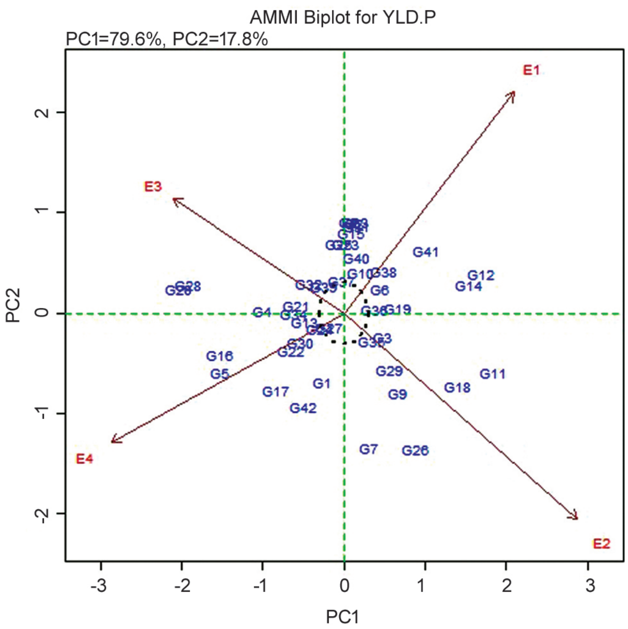

Fig 2 Gollob's F test, *** sig at P<0.001

AMMI Stability Value (ASV) and Yield Stability Index (YSI)

According to ASV ranking (Table 3), the landraces G35 (ILC184), G23 (IG5895), G25 (IG5904) has lowest ranking. These landraces performed well under both irrigated and rainfed condition. ASV parameter has been successfully used in several other studies to find stable genotypes (Sumathi *et al*. 2017). Stability *per se* is not a desirable selection criterion, because the most stable genotypes would not necessarily give the best performance (Mohammadi *et al*. 2007), hence, the ranking of ASV and mean performance taken in concordance as a single selection index, YSI. The lowest YSI indicates the most stable genotype with high mean performance.

Purchase *et al*. (2000) developed ASV which is quantitative measure to rank the genotypes through the AMMI model. It is most appropriate of describing the stability of genotypes over other parameters. Infact, ASV is the distance from zero in a two-dimensional scatter diagram of interaction PC1 scores against interaction PC2 scores. Because the Interaction PC1 score contributes more to SS for GE (Table 2), it must be weighted by the proportional difference between interaction PC1 and Interaction PCA2 scores to compensate. The distance from zero is then determined by using the theorem of Pythagoras (Purchase *et al*. 2000). The landraces G8 (IG5856), G41 (Pusa72), G21 (IG5884) and G9 (IG5858) were top ranked according to YSI (Table 3). The YSI was also used by many researchers to identify stable and well performing genotypes in chickpea (Kanouni 2018).

GGE BIPLOT

Mega environment delineation

GGE-biplot analysis indicated that the PC1 explained 80% and PC2 15.9% of variability, PC1 and PC2 together accounts for 95.9% of variability. The genotype with higher PC1 score means produce higher yield and stability of the genotype explained by PC2 score. The higher PC2 absolute value indicates instability of the genotype. PC score is the value of the line and environment coordinates displayed in the biplot curve. These coordinates were connected by line to form the polygon (Fig. 3). This polygon was divided into several sections called mega environments (Oliviera *et al*. 2010). What-when-where biplot (Fig 3) indicates that the polygon in this experiment is divided into seven sectors. The entire four environments were included in sector 1 and there are 11 genotypes which perform well under the entire four environments. The genotype G41 (Pusa 72) performs well under E1 and E2 and landrace G8 (IG 5856) performs well under all the 4 environments with highest yield.

Evaluation of environment

Fig 4 indicates the relationships between inter environment and inter-genotypes that can be shown by drawing a "straight stripe" from the origin of the biplot to the coordinates of the genotypes (Genotype vector) and the environment (Environmental vector), PC1 functions as the X-axis and PC2 as the Y-axis. The angle between E3 and E4 was less than 90^0indicate positive correlation between

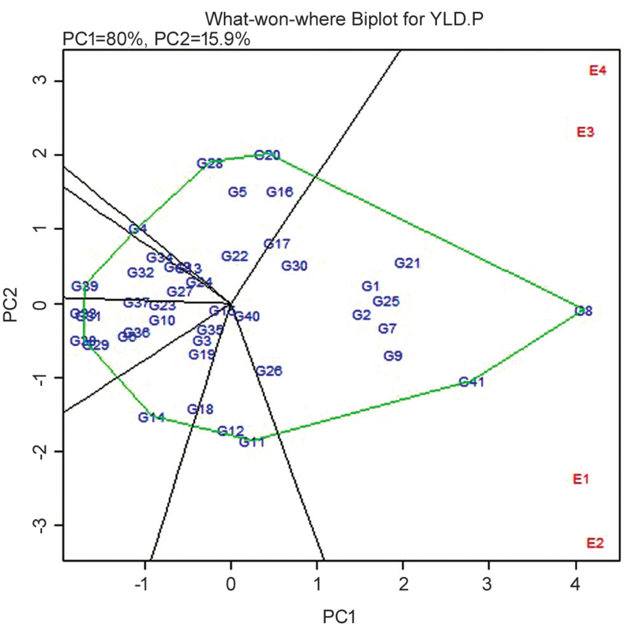

Fig 3 GGE biplot based on symmetrical scaling for 'which-won-where' pattern for the primary component of interaction (PC1) and second interaction principal component (PC2). Fig 2 AMMI2 first interaction principal components versus second principal component for chickpea landraces.

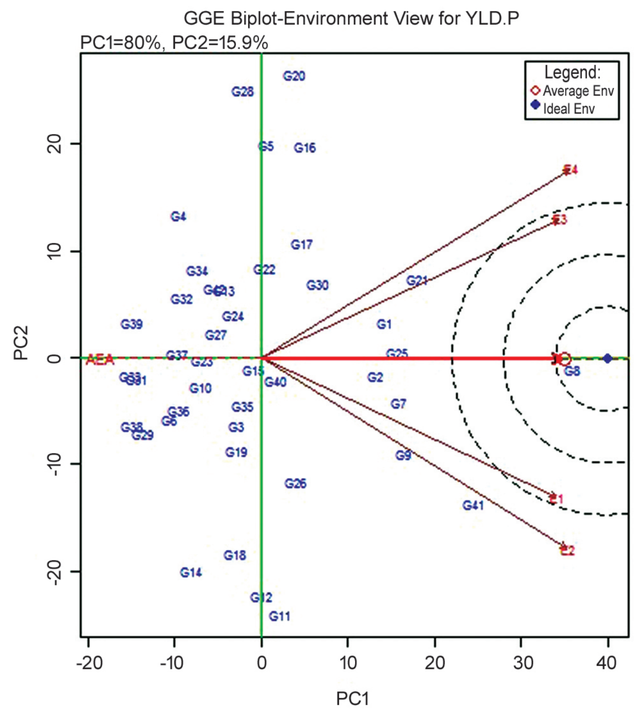

Fig 4 GGE-biplot environment view for yield that shows the correlation between test environments and correlation coefficient between any two environments is approximated by the cosine of the angle between heir vectors.

Table 3 The chickpea landraces with codes, mean yield, DSI, PC1, PC2, ranking of ASV and YSI

Code	Genotype	Means	DHAR DSI	IARI DSI	PC1	PC2	ASV	YSI	RASV	RSI
G1	ICC1882	19.54	1.0802	1.1374	19.5416	-0.1314	1.3726	21	14	7
G2	ICC4958	19.14	0.0630	0.0648	19.1488	0.0596	1.0437	17	9	8
G3	IG5842	11.37	1.3153	1.2732	11.3789	0.2391	2.176	44	21	23
G4	IG5843	8.2	1.8360	1.1488	8.2075	-0.4986	4.5127	67	33	34
G5	IG5844A	13.02	1.7320	1.3255	13.0200	-0.7510	6.8216	53	37	16
G6	IG5852	7.72	1.1475	1.3313	7.7275	0.2190	1.9959	56	19	37
G7	IG5855	20.35	1.4985	1.1452	20.3550	0.1494	1.9051	22	17	5
G8	IG5856	29.95	0.0335	0.0255	29.9538	0.0251	0.9373	8	7	1
G9	IG5858	20.63	1.0643	1.1646	20.6338	0.3303	3.0916	34	30	4
G10	IG5861	9.44	0.7751	0.9020	9.4400	0.1037	1.0217	38	8	30
G11	IG5862	13.79	1.3123	1.4388	13.7988	0.9062	8.2219	54	40	14
G12	IG5863	12.81	0.5936	0.9071	12.8106	0.8371	7.5861	57	39	18
G13	IG5864	10.69	1.4125	1.0381	10.6969	-0.2382	2.157	46	20	26
G14	IG5865	8.94	0.9056	1.3845	8.9406	0.7554	6.8422	70	38	32
G15	IG5866	12.42	0.1728	0.2836	12.4225	0.0439	0.8896	24	5	19
G16	IG5867	15.14	1.5774	0.7429	15.1438	-0.7498	6.7987	46	36	10
G17	IG5868	15	1.5201	1.3512	15.0076	-0.4094	3.783	42	31	11
G18	IG5870	11.29	1.5832	1.9715	11.2931	0.6917	6.3015	59	35	24
G19	IG5874	11.39	1.0267	1.0839	11.3998	0.3294	2.9814	51	29	22
G20	IG5878	14.56	0.5679	0.7424	14.5625	-1.0000	9.0533	55	42	13
G21	IG5884	21.37	0.4236	0.8158	21.3725	-0.2853	2.5832	29	26	3
G22	IG5886	12.93	1.2180	1.4861	12.9369	-0.3189	2.9097	45	28	17
G23	IG5895	9.49	0.3349	0.5730	9.4938	0.0102	0.7001	31	2	29
G24	IG5896	11.22	1.3750	1.0909	11.2263	-0.1483	1.3505	38	13	25
G25	IG5904	20.33	0.1708	0.2621	20.3300	-0.0268	0.7284	9	3	6
G26	IG5906	14.61	2.1060	0.6433	14.6175	0.4372	4.1812	44	32	12
G27	IG5909	10.26	1.1422	1.7184	10.2638	-0.0859	0.7897	31	4	27
G28	IG5980	11.74	0.4384	1.0153	11.7413	-0.9413	8.5232	62	41	21
G29	IG5985	6.18	2.3639	2.5842	6.1875	0.2740	2.5423	63	25	38
G30	IG5997	15.83	0.8391	1.3776	15.8300	-0.2666	2.4304	33	24	9
G31	ILC0(LATIVIA)	5.61	0.1851	0.2005	5.6188	0.0673	1.0895	52	10	42
G32	ILC1312	9.27	1.3458	1.3499	9.2725	-0.3072	2.7798	58	27	31
G33	ILC1313	11.76	1.2253	1.4993	11.7638	0.1673	1.5377	35	15	20
G34	ILC167	8.18	1.5587	0.9391	8.1875	0.1888	1.7085	51	16	35
G35	ILC184	8.16	0.9137	1.2652	8.1625	-0.0110	0.3409	37	1	36
G36	ILC1932	5.63	1.2448	1.1314	5.6375	0.2473	2.2766	63	22	41
G37	ILC239	5.65	1.4962	1.6127	5.6538	-0.1202	1.1209	51	11	40
G38	ILC8666	13.56	0.3993	0.5134	13.5675	0.0783	0.903176	21	6	15
G39	ILC0(CR)	5.9	0.1455	0.5091	5.9000	0.0801	1.1327	51	12	39
G40	ILC0(ITALY)	8.35	1.0122	1.1675	8.3545	-0.2097	1.9195	51	18	33
G41	PUSA72	24.57	0.1863	0.3436	24.5725	0.5032	4.5974	36	34	2
G42	SBD377	10.15	2.1508	2.0859	10.1525	-0.2441	2.3991	51	23	28

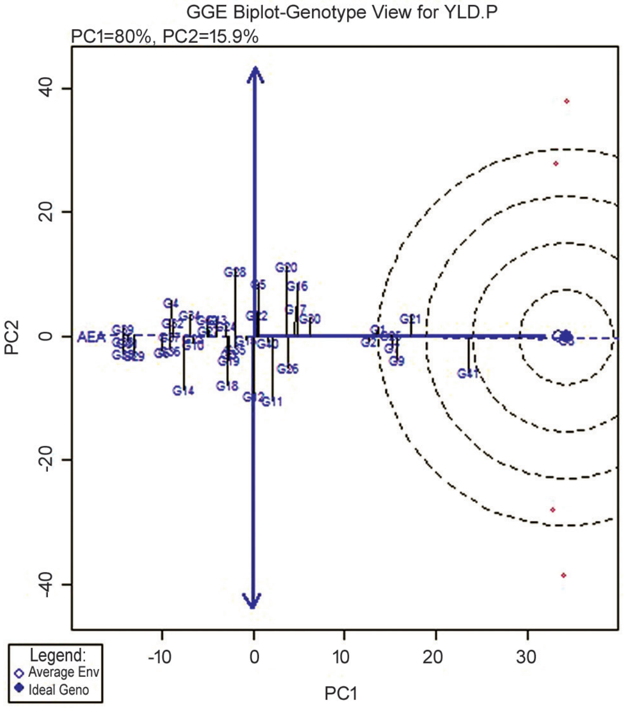

Fig 5 Visualization of GGE biplot showing the stability of genotypes, which linear line showing the axis of environment mean and interrupted circle is confidential range.

environments same results found between E1 and E2 (Yan and Tinker 2006). Average Environments Coordinates (AEC) point is the average coordinates of all environments located at the tip of the arrow in the Average Environment Axis (AEA) (Fig 4). Genotype at that point has an environmental interaction value equal to zero. The center of the circle on the AEA axis is the ideal environment for testing. The angle between AEA and E3 and AEA and E1 was less. It indicated that E3 (IARI irrigated) and E4 (IARI rainfed) was the ideal location to discriminate and show the performance of the tested genotypes.

Stability of genotypes

Fig 5 represents the average yield of each genotype illustrated by AEA axis and the stability of genotype illustrated by Y-axis, is a stripe perpendicular to the AEA axis. The higher yield indicated by the genotypes laid right of the Y-axis and shorter distance from AEA indicates stable genotypes. Genotypes that are in the concentric area were more stable in giving yield compared to the genotypes that were outside. The landraces G8 (IG5856) and G25 (IG5904) lay in the concentric area and on the AEA axis indicate that they are ideal landraces in terms of both yield and stability. The genotypes G41 (Pusa 72) and the land races G9 (IG 5858) and G21 (IG 5884) have shorter vector and this high yielding and are stable. The results are in concordance with those obtained from AMMI analyses. The ideal genotype is indicated by the point on the AEA axis in a positive position with the length from the center point of the biplot equal to the most extended genotype vector. The best genotype has the genetic distance closest to the ideal genotype point compared to other genotypes from Fig 5 we found that G8 (IG 5856) was the best genotype.

Conclusion

The results from AMMI and GGE analyses including DSI value shows that the landraces G8 (IG 5856) and G25 (IG 5904) perform well under all the environment, i.e. these genotypes are stable over all location and are drought tolerant. G8 (IG 5856) had the highest mean yield than the most preferred check variety G2 (ICC 4958) for drought tolerance. G23 (IG 5895) and G21 (IG 5884) were stable genotype but had lower mean yield. These landraces can be used in future chickpea pre-breeding programme for development of drought tolerant varieties with wider genetic base.

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