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IDENTIFICATION OF STABLE FORAGE SORGHUM GENOTYPES USING UNIVARIATE AND MULTIVARIATE ANALYSES

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SUMMARY

Genotype selection and recommendations are influenced by genotype x environment interactions (GEI). Identification of stable and high yielding cultivars is the main objective of the multi-environment data analysis. The objective of this study was to analyze rank correlations among ANOVA, Eberhart and Russell and biplot analyses in ranking of genotypes for yield, stability and yield-stability. The study included 16 forage sorghum genotypes evaluated at 14 environments across two years. The results showed significant effects due to environments, genotypes and GEI, suggesting differential response of genotypes. Environment (E) main effects accounted for >80% of the variation, compared to <20% for genotype (G) and GEI effects together. For yield rankings, all the three methods are positively and significantly correlated, while for stability ranking, ER and biplot analysis had positive significant correlation, indicating that both methods have identified the same genotypes for stability. GGE biplot has the advantage of identifying mega-environments and the genotypes for each mega-environment.

Key words : GGE biplot, Joint regression analysis, stability, yield ranking, correlations

Identification of high yielding and stable genotypes across variable environments has been a continued challenge to plant breeders worldwide. Frequent occurrence of genotype-by-environment interactions (GEI) often complicates testing and selection of superior genotypes thereby reducing genetic progress, Romagosa and Fox (1993).

Yan *et al.* (2000) developed a biplot technique named 'GGE biplot' which graphically represents the genotype(G) main effects plus GEI effects. It may be kept in mind that the measured value of each cultivar in a test environment is a cumulative measure of genotype main effect (G), environment main effect (E) and GEI, Yan *et al.* (2003). For evaluation of cultivar, both G and GE must be considered simultaneously, Yan *et al.* (2006); Sabaghnia *et al.* (2008). The G + GE (GGE) biplot removes the E and integrates the G with GEI effect of a G x E dataset, Yan *et al.* (2000). Effectively it detects the GEI pattern in the data and can identify 'which-won-where' besides identifying different mega environments, Yan *et al.* (2007). GGE biplot analysis has been carried out to understand GEI in many crop species and there are different reports on its utility in analysing and interpreting the complex GEI in MET data in case of grain sorghum, Rakshit *et al.* (2012), sweet sorghum, Rao *et al.* (2011) and forage sorghum, Aruna *et al.* (2015).

MATERIAL AND METHODS

Sixteen forage sorghum genotypes were evaluated across seven locations during the rainy seasons of 2010 and 2011 (total 14 environments). Detail features of the testing locations are given in Table 1. The testing locations were distributed across six states of India, with two locations in Gujarath and one each in Uttaranchal, Haryana, Punjab, Andhra Pradesh and Tamilnadu. Information on the genotypes used in the study is presented in Table 2. During both the years, the crops were sown during June-July depending on the onset of monsoon at the particular location. In each location, the experiment was conducted in randomized block design with two rows each of 4 m length with 45 x 10 cm^2 crop geometry. Crop management practices were standard across all locations. The plants were harvested at 50% flowering to estimate fodder yield.

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The fodder yield (FY) was recorded immediately after harvest to avoid moisture loss. For this, plants were harvested manually by cutting the stem at the base and the entire above ground plant material was weighed.

Statistical Analysis

ANOVA

Combined analysis of variance (ANOVA) for fodder yield data was performed to determine the effect of genotype (G), environment (E) and GEI using the model

$$Y_{ij} = \mu + G_i + E_j + GE_{ij}$$

Where Y_{ij} is the response variable of ith genotype at jth location, μ is the overall mean, G is the main effect of genotype i, E is the main effect of environment, j and GE is the error term but here it is confounded with GEI effect.

Eberhart and Russell Joint Regression Analysis

Linear regressions were carried out for each of the 16 genotypes based on the ER method. According to ER method, a regression coefficient (slope) = 1 and variance deviation (var-dev) = 0 indicates stability. In this analysis, var-dev is the error mean square (EMS) of regression analysis. The ANOVA and joint regression analyses were performed using Windostat software.

Variance in regression deviation (S^2di) was calculated, as suggested by, Eberhart and Russell, (1966).

$$Y_{ij} = \mu + G_i + E_j + b_iEj + d_{ij} + e_{ij}$$

Where Y_{ij} is the mean yield for the i^{th} genotype in the j^{th} environment; μ is the grand mean; G_i is the effect of genotype i (i= 1,2,…..,g) and E_j is the effect of environment j (j = 1, 2, ……, e); bi is the linear regression coefficient of the i^{th} genotype on environmental index; d_{ij} is deviation from regression; and e_{ij} is the average of the random errors associated with the i^{th} genotype and j^{th} environment.

GGE Biplot Analysis

GGE biplot methodology, consisting of two concepts, the biplot concept, Garriel *et al.* (1971) and the GGE concept, Yan *et al.* (2000), was used to analyse the data. The statistical theory of GGE methodology has been explained in detail by, Yan and Kang, (2003). The MLT (multi-location trial) data was analyzed as described by, Rakshit *et al.* (2012) using the software GGE biplot ver.6.3, Yan *et al.* (2001). The MLT data was analyzed without scaling ('Scaling 0' option) to generate a tester centered (centering 2) GGE biplot as suggested by, Yan *et al.* (2006). For genotype evaluation, genotype focused singular value partitioning (SVP=1) was used using the 'Mean versus stability' option of GGE biplot software, Yan (2001). 'Which-won-where' option was used to identify which genotype was the winner in a given set of environments and to identify mega-environments.

Correlation Analysis Among Different Statistical Methods

Spearman's rank correlation coefficients were determined among the ranks given by each of the statistical methods *i. e.*, ER, GGE biplot and ANOVA analyses. For each statistical method three kinds of ranks (yield ranks, stability ranks and yield-stability ranks) were determined. The yield ranks in ER method were determined by giving the best rank to the genotype having the highest regression coefficient and the last rank was given to the genotype having lowest regression coefficient, Mohammadi and Amri (2013).

TABLE 1
Information on the study environments

Location	Code	Longitude	Latitude	Elevation (msl)	Average rainfall (mm)
Pantnagar, Uttaranchal	L1	79.52°E	29.05°N	243.8 mt	1450
Deesa, Gujarath	L2	72.18°E	24.26°N	148 mt	889
Coimbatore, Tamilnadu	L3	76.97°E	11.02°N	411 mt	693
Surat, Gujarath	L4	72.49°E	21°10°N	13 mt	1200
Hisar, Haryana	L5	75.72°E	29.15°N	215 mt	450
Ludhiana, Punjab	L6	75.85°E	30.91°N	244 mt	733
Hyderabad, Andhra Pradesh	L7	78.49°E	17.39°N	536 mt	812.5

TABLE 2
Information on the genotypes used in the study

Genotype	Code	Characteristics
Improved Ramkel	G1	Selection from a forage local from Maharashtra
GFS5	G2	Improved forage line from Gujarath
S541	G3	Improved forage line from Haryana
Rampur local	G4	Local germplasm line from UP
MP Chari	G5	Improved forage line from MP
COFS29	G6	Improved forage line from Tamilnadu
PSC1	G7	Early maturing forage line from Punjab
SL44	G8	Early maturing forage line from Punjab
Katarkhatav	G9	Local germplasm line from Maharashtra
Sangolahundi	G10	Local germplasm line from Maharashtra
HC308	G11	Nationally released forage line
CSV21F	G12	Nationally released forage line
SSG59-3	G13	Nationally released forage line
SSV84	G14	Nationally released sweet sorghum line
CSV19SS	G15	Nationally released sweet sorghum line
SSV74	G16	Nationally released sweet sorghum line

To obtain GGE biplot yield ranks, the best rank was given to the ideal genotype which is on the far right hand side and the last rank was given to the genotype on the far left hand side of the biplot. The ANOVA yield ranks were obtained from the phenotypic yield data.

The ER stability rankings were obtained by allotting best rank to the genotype with lowest S^2di. The ER yield-stability ranks were then determined as the sum of ER yield and stability ranks. The GGE stability rankings were determined as visual ratings on the projections of genotypes on the AEC (average environment coordinate): a smaller projection equated to a better stability ranking. The GGE yield-stability rankings were then determined as the sum of GGE yield and stability rankings. The ANOVA stability rankings were calculated by obtaining average of the ranks of each genotype across 14 environments. The ANOVA yield-stability rankings were determined as the sum of ANOVA yield and stability rankings.

RESULTS AND DISCUSSION

The ANOVA results are presented in Table 3, which indicated that the environment (E) and genotype (G) main effects and GEI were significant implying a substantial variation among the genotypes as well as environments. The highest percentage of variation was explained by E main effect (94.4% in 2010; 88.3% in 2011 and 84.0% in combined analysis), while G and GE effects together explained the rest of the variation.

ER Joint-Regression Analysis

The ER regression analysis results are presented in Table 4. According to ER method, the genotypes with high regression coefficients are considered as high yielding genotypes and those with low regression coefficients as low yielding. At the same time, the genotypes with high s^2di estimates are considered as highly unstable and those with low s^2di estimates as highly stable. In this study, COFS 29 (G6), SSG 59-3 (G13) and CSV 21F (G12) are judged as high yielding whereas CSV 19SS (G15), PSC 1 (G7) and Sangolahundi (G10) were regarded as low yielding genotypes. The genotypes SSG 59-3 (G13), CSV 19SS (G15), Rampur local (G4), Improved Ramkel (G1) and SL 44 (G8) were considered top 5 stable lines. On the otherhand the genotypes, COFS 29 (G6), S 541 (G3), CSV 21F (G12), SSV 74 (G16) and Katar khatav (G9) were the top 5 unstable genotypes. Based on the combined information of regression coefficient and s^2di estimates, the genotype SSG 59-3 (G13) was judged to be both high yielding and highly stable.

GGE Biplot Analysis

The GGE biplot explained 78.5% of total variation with PC1 and PC2 accounting for 58.8% and 19.7%, respectively (Fig. 1). The genotypes, COFS 29 (G6), CSV 21F (G12) and SSG 59-3 (G13) were top ranking as they are present on the far right hand side of the biplot towards the pointing arrow of the AEC abscissa (Fig.1). The biplot indicates that the genotypes SSV 84 (G14), SSG 59-3 (G13) and SL 44 (G8) are highly stable as they are positioned very near to the AEC abscissa with near zero scores. In contrast, the highest yielding genotype, COFS 29 (G6), is deemed to be unstable as it has a very high PC2 score and is away from AEC abscissa.

ANOVA Analysis

The per se yield performance indicates that COFS 29 (G6) is the highest yielder followed by CSV 21F (G12) and SSG 59-3 (G13). For stability, the same 3 genotypes, SSG 59-3>CSV 21F>COFS 29 were found to be good (Table 4).

Rank Correlation Analysis Among the Statistical Methods

Spearman's rank correlations among the three statistical methods based on yield ranks, stability ranks and yield-stability ranks are given in Table 5. The yield rank correlations among the three methods were observed to be positive and significant indicating that all these methods identified same genotypes for yield superiority. The correlations between ER method and ANOVA was found to be 0.54 whereas between GGE biplot and ANOVA it was found to be 0.82 (Table 5) indicating that the GGE biplot results are better reflecting ANOVA results than those from ER analysis. The yield rank correlation between GGE biplot and ER method (r= 0.67), however, suggests that the two methods detected common genotypes that were either high yielding and/or low yielding. For example, the top three hybrids based on ANOVA, ER method and GGE biplot are the same (Table 6).

The stability ranking of GGE biplot was found to be significantly and positively correlated with ER stability rankings, while the correlation of ANOVA stability ranks with those of GGE and ER were negative and non-significant, indicating that assessing stability based on *per se* performance may not be appropriate. Four genotypes among the top five most stable genotypes were common between GGE biplot and ER methods (Table 6).

The yield-stability correlations between GGE biplot ranks and ER ranks; and between GGE ranks and ANOVA ranks were observed to be significant, while that between ER ranks and ANOVA ranks was non-significant. Considering yield and stability, the

TABLE 3
ANOVA and proportion of variation (G+E+GE) explained by genotype (G), environment (E) and GEI for fodder yield

Year		Source		
		G	E	GEI
2010	MS	109116.6**	2497897**	39091.03**
	Proportion (%)	4.1	94.4	1.48
2011	MS	96847.2**	1060524**	43565.9**
	Proportion (%)	8.06	88.3	3.63
Combined	MS	196903.7**	1397169**	70268.02**
	Proportion (%)	11.83	83.95	4.22

TABLE 4
Mean yield, yield ranks and stability ranks using different statistical methods for 16 genotypes tested in 14 environments

Genotypes	Per se performance			E-R model			GGE biplot		
	Mean yields (q/ha)	Yield ranks	Stability ranks	Regression coefficient	Rank	S^2di	Rank	Yield rank	Stability rank
Improved Ramkel	467.1	4	4	0.99	8	1870.5	4	5	4
GFS 5	418.0	9	8	0.94	11	6073.9	9	13	10
S 541	450.9	5	10	1.11	4	25547.3	15	4	15
Rampur local	405.9	12	10	1.08	5	1596.6	3	8	5
MP Chari	394.6	13	11	1.05	7	1943.7	5	12	6
CO FS 29	623.5	1	3	1.30	1	57146.4	16	1	16
PSC 1	351.3	15	13	0.89	15	1038.1	1	14	9
SL 44	341.9	16	14	1.05	6	5536.8	8	16	3
Katarkhatav	416.2	10	9	0.95	9	7577.2	12	10	12
Sangolahundi	435.8	8	8	0.93	14	4935.9	7	7	8
HC 308	445.2	6	7	0.93	12	6345.4	10	6	13
CSV 21F	524.3	2	2	1.12	3	20142.0	14	2	14
SSG 59-3	485.1	3	1	1.18	2	2084.0	6	3	2
SSV 84	377.7	14	12	0.93	13	6543.6	11	15	1
CSV 19SS	406.2	11	6	0.59	16	1431.1	2	11	7
SSV 74	436.6	7	5	0.95	10	9066.8	13	9	11

genotypes G13, G1 and G4 were identified promising by both ER method and GGE biplot method (Table 6). Among the genotypes, SSG 59-3 was found to be high yielding and stable using the three statistical methods.

The GGE biplot, in addition, provides a "which won where" polygon view of genotypes to identify genotypes potentially suited to specific mega-environments. In this view the vertex genotype in each sector is considered the best genotype suited to those environments that fall into the respective sectors. In broader terms, the sites within the same sector of a polygon share the same best genotype. Which-won-where biplot for fodder yield is presented in Fig. 2. The biplot indicated existence of crossover GE and existence of mega-environments. The polygon had four genotypes, viz. G6, G3, G14 and G2 at the vertices. The equality lines divided the biplot into three sectors, of which two retained all seven locations. Thus the testing locations may be partitioned into two mega-environments: one with L1, L2, L3, L4 and L6 with G6 as the winning genotype. Second mega-environment encompassed L5 and L7 with G3 as the winning genotype, Aruna *et al.* (2015).

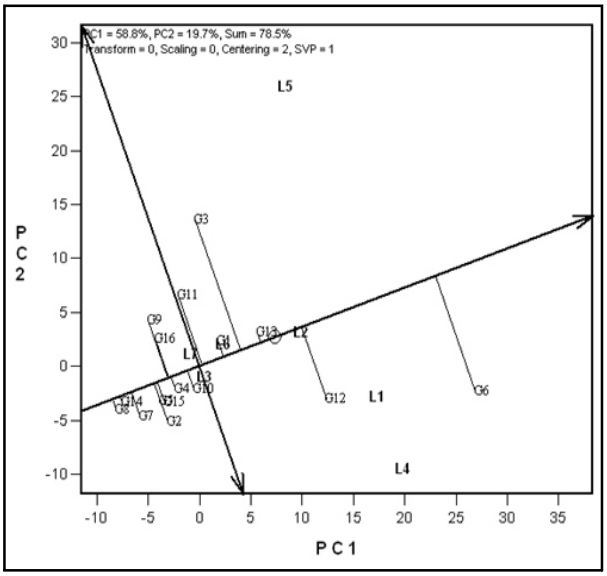

Fig. 1. GGE biplots of the combined analysis indicating mean vs stability of the genotypes for Fodder yield.

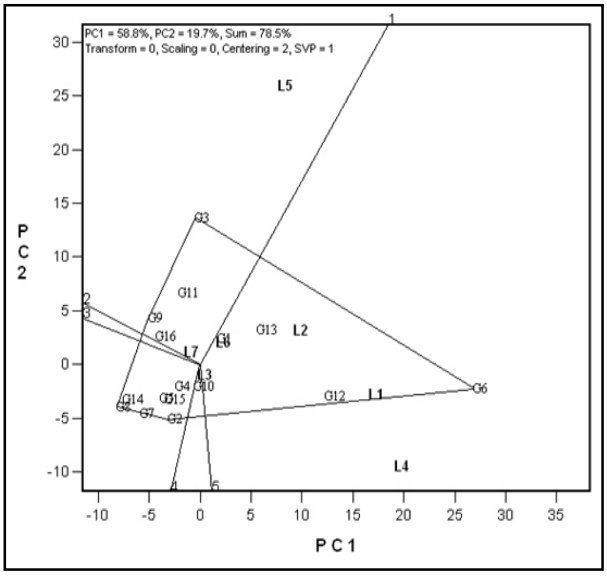

Fig. 2. GGE biplot indicating which-won-where analysis of the genotypes for fodder yield combined over years.

TABLE 5
Yield, stability and yield-stability correlations among GGE biplot, Eberhart-Russell (ER) and analysis of variance (ANOVA) ranks

		E-R ranks	ANOVA ranks
Yield	GGE ranks	0.67**	0.82**
	ANOVA ranks	0.54*	
Stability	GGE ranks	0.69**	-0.26
	ANOVA ranks	-0.26	
Yield-stability	GGE ranks	0.53*	0.50*
	ANOVA ranks	0.18	

GGE biplot view besides indicating the presence (or absence) of GEI, suggests the presence or absence of different mega environments which cannot be identified in ER method. Although the "which won where' view seems to be ideal for exploiting the GE interactions, caution has to be taken while recommending genotypes to specific locations because sometimes the variation explained by the PC1 and PC2 scores of the GGE model might be too low. In the GGE biplot analysis, if the first two PC explain more than 60% of the variability in the data, and the combined G+GE effect account for more than 10% of the total variability, then the biplot adequately approximates the variability in the MET data, Yang *et al.* (2009); Yan *et al.* (2010). In our study the first two PC explained more than 70% of the variability and G + GE explained >10% of variability. Thus, the obtained biplot can be used effectively to interpret the variability in the MLT data.

GGE biplot has additional merits in providing more information and comprehensive visualization of the location patterns that allow greater discrimination among genotypes and their relationships between environments which are not obtainable from ER method, Alwala *et al.* (2010). Moreover, GGE biplots play a vital role in selecting superior genotypes in early stages of testing where a large number of genotypes is being evaluated in very few locations and when the main objective is to discard the inferior genotypes.

TABLE 6
Top five genotypes for yield, stability, yield-stability by different statistical methods

ANOVA (per se performance)			ER method			GGE biplot anlaysis		
Yield	Stability	Yield stability	Yield	Stability	Yield stability	Yield	Stability	Yield stability
COFS 29	SSG 59-3	COFS 29	COFS 29	SSG 59-3	SSG 59-3	COFS 29	SSV 84	SSG 59-3
CSV 21F	CSV 21F	CSV 21F	SSG 59-3	CSV 19SS	Rampur local	CSV 21F	SSG 59-3	Improved Ramkel
SSG 59-3	COFS 29	SSG59-3	CSV 21F	Rampur local	SL 44	SSG 59-3	SL 44	Rampur local
Improved Ramkel	Improved Ramkel	Improved Ramkel	S 541	Improved Ramkel	Improved Ramkel	S 541	Improved Ramkel	Sangolahundi
S 541	SSV 74	SSV 74	Rampur local	SL 44	MP Chari	Improved Ramkel	Rampur local	CSV 21F

CONCLUSION

Identifying stable and high yielding genotypes is the most ideal way to avoid GEI. The results indicated the presence of significant variability among genotypes, environments and their interaction. In this study, it was evident that the GGE mode produced a clear distinction among the genotypes with regard to their yields and stability. All the three statistical methods identified the same genotypes as far as yield is concerned. While in identifying stable genotypes, ER and GGE biplot methods were more related. The GGE biplot portrayed the genotypes based on their yields as well as their stabilities in a two dimensional display. Considerable genotypic differences in yield response to divergent environments in this study, suggest that a systematic effort is needed to screen different genotypes across different environments to identify those that perform well across or within or a specific target region of environments.

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