CrossMark

RESEARCH ARTICLE

Identification of Ideal Locations and Stable High Biomass Sorghum Genotypes in semiarid Tropics

G. S. Anil Kumar[1] · K. S. Vinutha[1] · Devendra Kumar Shrivastava[2] ·
S. Jain[2] · B. A. Syed[3] · B. Gami[3] · S. Marimuthu[4] · A. Yuvraj[4] · H. S. Yadava[2] ·
S. C. Srivastava[2] · K. Yadagiri[5] · V. Ansodariya[6] · P. Prasuna[7] · J. Vishwanath[8] ·
S. R. Anand[8] · Abhishek Rathore[1] · A. V. Umakanth[5] · P. Srinivasa Rao[1,9]

Abstract The dearth of proper delineation for energy sorghum cultivation has led to a prerequisite for evaluation and identification of test environments for the newly developed lines. This becomes of vital importance as the biomass yield is highly influenced by genotype and environmental (G × E) interactions. Several agronomic traits were considered to assess the biomass yield and the combined analysis of variance for G (genotype), L (location) and interaction effect of G × L. The variations in the yield caused by the interaction of G × L are very essential to acquire knowledge on the specific adaptation of a genotype. Thus, the multi-location trials conducted across locations and years have helped to identify the stable environments with specific adaptation for biomass sorghum. The presence of close association between the test locations suggested that the same information about the genotypes could be obtained from fewer test environments, and hence the potential to reduce evaluation costs. The two genotypes—IS 13762 and ICSV 25333—have shown stable performance for biomass traits across all the locations, in comparison with CSH 22SS (check). The top ten entries with stable and better performance for fresh biomass yield, dry biomass yield, grain yield and theoretical ethanol yield were ICSV 25333, IS 13762, CSH 22SS, IS 25302, IS 25301, IS 27246, IS 16529, DHBM2, ICSSH 28 and IS 17349.

Keywords Biomass · Energy sorghum · GGE biplot · Stability · Rainfed tropics · Ethanol yield

✉ P. Srinivasa Rao
psrao72@gmail.com

[1] Sorghum Breeding, International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad, Telangana 502 324, India

[2] Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior, Madhya Pradesh 474 002, India

[3] Abellon Cleanenergy Limited, Ahmedabad, Gujarat 380 054, India

[4] Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu 641 003, India

[5] Indian Institute of Millet Research, Hyderabad, Telangana 500 030, India

[6] Junagadh Agricultural University, Jamnagar, Gujarat 362 015, India

[7] Agricultural College, Acharya N. G. Ranga Agricultural University, Bapatla, Andhra Pradesh 522 101, India

[8] Agricultural Research Station, Gangavathi University of Agricultural Sciences Raichur, Raichur 583 227, India

[9] Present Address: Florida A&M University (FAMU), Tallahassee, FL 32317, US

Abbreviations

FSY	Fresh biomass yield
DSY	Dry biomass yield
GY	Grain yield
GGE	Genotype main effect plus genotype by environment interaction
PC	Principal component
ICRISAT	International Crop Research Institute for the semiarid Tropics
IIMR	Indian Institute of Millets Research
PCA	Principal component analysis
RCBD	Randomized complete block design
MLT	Multi-location trial
G	Genotype
E	Environment

L   Location
TEY   Theoretical ethanol yield

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world, cultivated in the arid and semiarid tropics (SAT) for its better adaptation to various stresses, including drought, heat, salinity and flooding (Ejeta and Knoll 2007). The sorghum crop, cultivated as a biomass feedstock, is often referred as "Energy or High biomass" sorghum (Meki et al. 2013), which is characterized by 3–4 m tallness and completing lifecycle in over 120 days. The dry biomass accumulated is double that of grain sorghum (Olson et al. 2012), and the biomass yield varies between 15 and 25 t ha^{-1} and yields as high as 40 t ha^{-1} (Packer and Rooney 2014). Trials of energy sorghum hybrids ranged from 27.2 to 32.4 t ha^{-1} (Packer and Rooney 2014). Therefore, the crop can be used as a dedicated bioenergy crop (Gill et al. 2014) as the potentiality of sorghum by crop modeling estimations shows that the biomass accumulation was colossal (Olson et al. 2012). A higher stem-to-leaf biomass ratio (Olson et al. 2012, . 2013; Packer and Rooney 2014; Gill et al. 2014) was also observed, and the shoot biomass recorded was 83% higher than grain sorghum due to longer growth duration (Olson et al. 2012), and very efficient nitrogen remobilization from lower leaves and stem internodes during development (Olson et al. 2013). The energy sorghum has superior agronomic traits such as large stem girth (18–23 mm) and high lodging tolerance. Thus, utilization of high biomass yielding sorghum will reduce the competition for land utilization with food crops (Olson et al. 2012) and helps in bioremediation as sorghum is cultivated in degraded lands owing to its innate nature of drought tolerance and moderate tolerance to salinity stress (Sathya et al. 2016).

In the process of conducting multi-location trails (MLT) for new cultivars, major emphasis is given on the agronomic superiority of the new cultivars over the ruling cultivars in terms of gain in grain and or biomass and little or no emphasis is given on interaction of the cultivars with the target environments, which is mostly unpredictable (Rakshit et al. 2012). The performance of a genotype varies under different environmental conditions, and thus reduction in inheritability of yield and its attributing traits and in turn reduction in genetic gain (Matheson and Raymond 1986) was observed. The genotype main effect plus genotype-by-environment interaction (GGE) varies the usefulness of genotypes by varying their yield performance (Pham and Kang 1988) through minimizing the association between genotype and phenotype (Comstock and Moll 1963). It also helps to select genotypes for higher yield stability within relatively well-defined and homogeneous environments and increases the efficiency of breeding programs by targeting genotypes to appropriate production areas (Brown et al. 1983; Peterson and Pfeiffer 1989).

One of the latest statistical method which is in use for genotype × environment (G × E) data analysis is the genotype + (genotype × environment) interaction (GGE) biplots (Yan and Kang 2003; Yan and Tinker 2006). Plant breeders have found GGE biplots to be useful in the evaluation of test environments (Yan and Rajcan 2002; Blanche and Myers 2006; Thomason and Phillips 2006; Srinivasa rao et al. 2011). The GGE provides both additive and multiplicative effects represented by principal component analysis (PCA) (Yan et al. 2000). The variation in yield is high among different genotypes, but the year × location, location × season, year × location × season is considerable (Rakshit et al. 2012; Olson et al. 2013; Gill et al. 2014). The selection process will be complicated with the presence of GGE.

The ethanol production is being extensively carried by starch and cellulosic materials; recently, deployment of lignocellulosic materials for ethanol production is a growing trend where aboveground plant part is completely utilized. Plant biomass, post-harvesting is a humungous leftover resource in field, and ethanol produced from biomass has low CO_2 emissions. Consequently, the biomass has been recognized as the promising feedstock for ethanol production (Berndes et al. 2003; Antonopoulou et al. 2008). Sorghum biomass contains 22.6–47.8% insoluble cellulose and hemicellulose (Dolciotti et al. 1998; Rattunde et al. 2001; Antonopoulou et al. 2008) making itself more amenable for lignocellulosic conversion. However, plants like energy sorghum capable of accumulating biomass under marginal lands with substantial yield loss can also be diverged for ethanol production. This will, as well avoid the competition with food crops and fertile lands for cultivation, although a higher net return from ethanol production would tend the farmer to dedicate the fertile lands to increase biomass yield. The ethanol yield is calculated from the dry biomass yield, cellulose and hemicellulose content (Rinne et al. 1997; Institution of Japan Energy 2006; Zhao et al. 2009), the predicted ethanol yield helps in scaling up and identify the viable genotypes for commercial scale distillery operations. Therefore, sorghum has been considered as an important feedstock for fuel ethanol production (Mamma et al. 1995; Buxton et al. 1999).

Thus, the present investigation is focused on the evaluation of the newly generated materials for biomass production, including nationally released varieties as checks through multi-location trials in rainfed sorghum growing tropical regions of India and stable entries across the

location were used for evaluating the theoretical ethanol yield. The important measure for testing of environments is the discriminating power of GGE biplot (Dehghani et al. 2006). It shows the discriminating ability of the environments and also helps to visualize the length of the environment vectors proportionate to standard deviation within the respective environments on the biplot (Yan and Tinker 2006).

Materials and Methods

Test Entries, Testing Locations and Crop Cultivation

The materials used in the study consisted of 65 biomass sorghum genotypes, 40 in 2013 and 25 in 2014 bred from International Crops Research Institute for the semiarid Tropics (ICRISAT), Indian Institute of Millet Research (IIMR) and National Agriculture Research System (NARS) partners representing various kinds of sorghum grown in India (Table 1). The experiment design adopted was randomized complete block design (RCBD) with three replications in rainy season of both the years. The plot size was 4 rows × 4 m spaced 0.6 m apart, and plant to plant was 12 cm. Seed treatment against soil borne pests and diseases was performed with thiram 3 g kg^{-1} seed. Seedlings were thinned to one plant per hill after 3 weeks from sowing. To ensure experimental uniformity and reduce the errors, sowing and management practices across the locations were followed uniformly in both the season.

The test locations in the study were chosen from four states across India (Fig. 1), accounting for total 19 locations (Table 2), 10 in 2013 and 9 in 2014. Of the 11 locations across 2 years, trials in eight locations were conducted in both the years and only once in three locations. These locations are the representative areas of the rainfed tropics. Due to the scarcity of precise phenotyping of the energy sorghum genotypes, the lines with biomass

locations are the representative areas of the rainfed tropics in sorghum growing regions of India.

Data Collection

The data on days to 50% flowering were recorded at anthesis and upon maturity, the middle two rows were harvested, and, plant height (m), stem girth at 3rd and at 10th internode (mm), number of internodes, fresh biomass yield (t ha^{-1}), dry biomass yield (t ha^{-1}) and grain yield (t ha^{-1}) were recorded (Nagaiah et al. 2012). For measuring the plant height rulers were used, and the stem girth was recorded using digital vernier calipers. The biomass was harvested from the middle two rows to record the fresh biomass weight, later sun dried for about 3 weeks, and the dry weights were recorded. For analyzing GGE in biplot, the fresh biomass yield (t ha^{-1}), dry biomass yield (t ha^{-1}) and grain yield (t ha^{-1}) data were used in the study.

Biomass Composition Analysis and Theoretical Ethanol Yield (L ha^{-1})

Ten biomass sorghum plants per plot in the three replications (biological sampling) were sampled for stover fodder quality analysis, mechanically chopped and dried at 60 °C for 5 days and then ground to pass through a 1 mm mesh. Samples were analyzed using near-infrared spectroscopy (NIRS; Instrument FOSS 5000 Forage Analyzer with WINSI II software package) in two technical replicates. Nitrogen (N), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and in vitro organic matter digestibility (IVOMD) were investigated (Bidinger and Blümmel 2007; Blümmel et al. 2007, 2015) and for good-of-fitness of the developed NIRS calibration model see Blümmel et al. (2003). Based on the analysis cellulose and hemicellulose were derived (Rinne et al. 1997; Zhao et al. 2009), further theoretical ethanol yield (TEY) was calculated by using the below given formula (Rinne et al. 1997; Institution of Japan Energy 2006; Zhao et al. 2009).

$$\text{Theoretical ethanol yield (L ha}^{-1}) = \frac{(\text{cellulose} + \text{hemicellulose } (\%)) \times \text{dry biomass (t ha}^{-1}) \times 1.1 \times 0.8 \times 0.5 \times 0.8 \times 1000}{0.7 \times 100}$$

yield above 20 t ha^{-1} was used as a selection criterion from ICRISAT and IIMR breeding materials for evaluation. Moreover, the genotypes used in each trial were different as new genotypes were included in second year. The

Statistical Analysis

Combined analysis of variance across different locations was performed to test the significance of main and

**Table 1** List of sorghum genotypes used for conducting multi-location trials

G. no.	Genotype	
1	CMSXS633	Imported line from Brazil
2	CMSXS645	Imported line from Brazil
3	Gird 36	Local cultivar from Madhya Pradesh
4	Gird 8	Local cultivar from Madhya Pradesh
5	HBM-1071	Selection from high biomass population
6	IS 17248	ICRISAT germplasm accession
7	ICSV 25335	High biomass variety from ICRISAT
8	IS 18542	ICRISAT germplasm accession
9	IS 25298	ICRISAT germplasm accession
10	IS 25301	ICRISAT germplasm accession
11	IS 25302	ICRISAT germplasm accession
12	ICSV 25333	High biomass variety from ICRISAT
13	IS 27246	ICRISAT germplasm accession
14	IS 38024	ICRISAT germplasm accession
15	N 610	*bmr* mutant line
16	SelB-Pop.	Selection from sorghum population at ICRISAT
17	DHBM-1	Advanced breeding derivative from NARS
18	DHBM-2	Advanced breeding derivative from NARS
19	DHBM-3	Advanced breeding derivative from NARS
20	DHBM-4	Advanced breeding derivative from NARS
21	DHBM-5	Advanced breeding derivative from NARS
22	DHBM-6	Advanced breeding derivative from NARS
23	CSH 22SS	National sweet sorghum hybrid check
24	ICSV 93046	Sweet sorghum variety from ICRISAT
25	SSG 59-3	National forage sorghum varietal check
26	COFS 30	Tamil Nadu State forage sorghum varietal check
27	COFS 31	Tamil Nadu State forage sorghum varietal check
28	CSH 24MF	National forage sorghum hybrid check
29	ICSSH 28	Dual purpose hybrid-sweet/biomass sorghum
30	ICSSH 75	Dual purpose hybrid-sweet/biomass sorghum
31	Vidisha 60	Local cultivar from Madhya Pradesh
32	Chohatia	Local cultivar from Gujarat
33	CMSXS641	Imported line from Brazil
34	CSH 13	National dual purpose hybrid check
35	CSV 24SS	National sweet sorghum varietal check
36	Gwalior I	Local cultivar from Madhya Pradesh
37	Gwalior II	Local cultivar from Madhya Pradesh
38	Gwalior III	Local cultivar from Madhya Pradesh
39	IS 13526	ICRISAT germplasm accession
40	IS 13540	ICRISAT germplasm accession
41	IS 13554	ICRISAT germplasm accession
42	IS 13762	ICRISAT germplasm accession
43	IS 15957	ICRISAT germplasm accession
44	IS 16527	ICRISAT germplasm accession
45	IS 16529	ICRISAT germplasm accession
46	IS 16575	ICRISAT germplasm accession
47	IS 16611	ICRISAT germplasm accession
48	IS 17349	ICRISAT germplasm accession
49	IS 21893	ICRISAT germplasm accession

Table 1 continued

G. no.	Genotype	
50	IS 22670	ICRISAT germplasm accession
51	IS 22868	ICRISAT germplasm accession
52	IS 22879	ICRISAT germplasm accession
53	IS 23101	ICRISAT germplasm accession
54	IS 23120	ICRISAT germplasm accession
55	IS 25186	ICRISAT germplasm accession
56	IS 25234	ICRISAT germplasm accession
57	IS 26204	ICRISAT germplasm accession
58	IS 27206	ICRISAT germplasm accession
59	IS 6750	ICRISAT germplasm accession
60	IS 8813	ICRISAT germplasm accession
61	MP I	Local cultivar from Madhya Pradesh
62	MP II	Local cultivar from Madhya Pradesh
63	MP III	Local cultivar from Madhya Pradesh
64	SPSSV 30	Advanced breeding derivative
65	SSV 74	Sweet sorghum varietal check

G. no. genotype number

Fig. 1 Testing locations chosen for conducting multi-location trials across four states in India (📍—state; white dot—test locations)
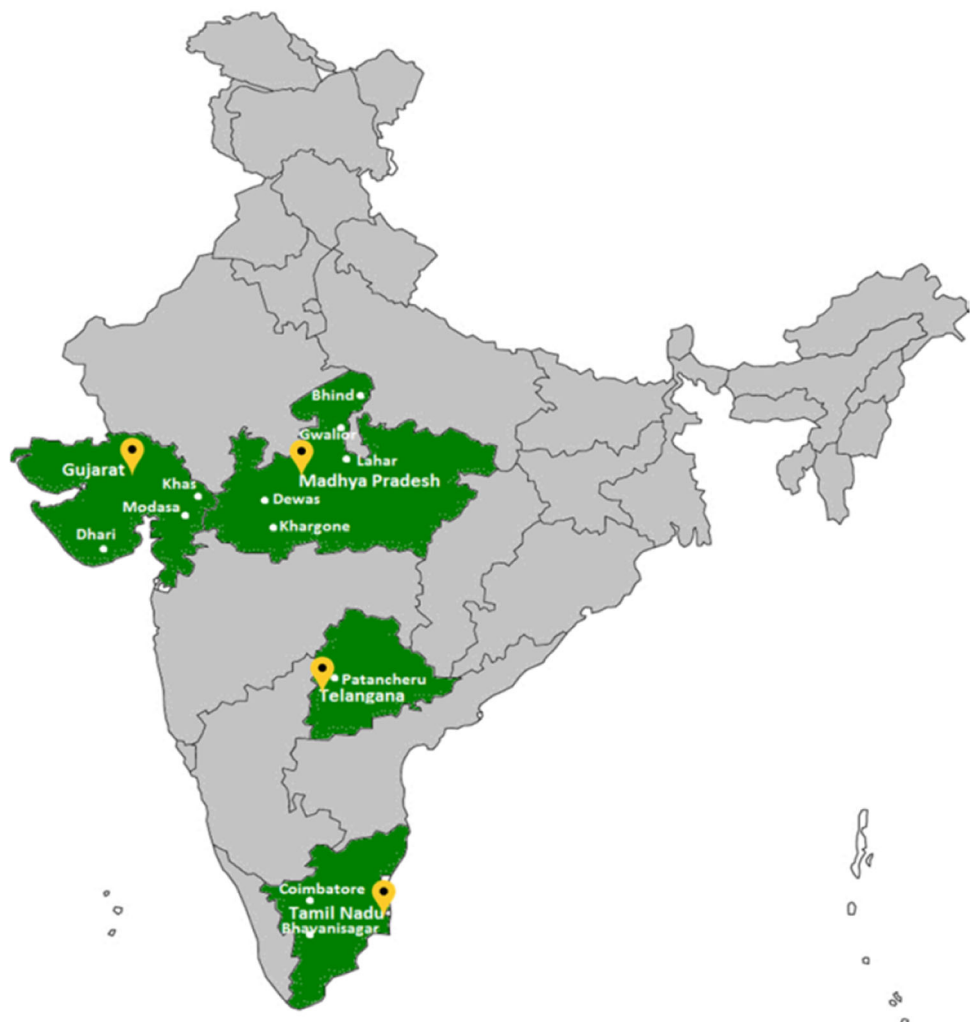
Table 2 Test environments used in the multi-location trials selected from the four states

Sl no.	Environment code	Environment name	Latitude and longitude	Rainfall (mm)	State
1	BVR_2013	Bhavanisagar	11°28′30.3″N	717.0	Tamil Nadu
2	BVR_2014	Bhavanisagar	77°07′55.9″E		
3	COI_2013	Coimbatore	11°00′14.2″N	689.3	
4	COI_2014	Coimbatore	76°57′08.3″E		
5	DVS_2013	Dewas	22°57′41.6″N	924.1	South Madhya Pradesh
6	DVS_2014	Dewas	76°03′25.5″E		
7	KRG_2013	Khargone	21°59′49.4″N	731.8	
8	KRG_2014	Khargone	76°00′24.2″E		
9	GWR_2013	Gwalior	26°17′22.5″N	776.3	North Madhya Pradesh
10	GWR_2014	Gwalior	78°09′42.8″E		
11	LHR_2013	Lahar	26°11′41.3″N	806.2	
			78°56′38.8″E		
12	BND_2014	Bhind	26°24′31.8″N	667.8	
			78°42′34.8″E		
13	ICRISAT_2013	Patancheru	17°31′02.5″N	1074.2	Telangana
14	ICRISAT_2014	Patancheru	78°16′44.9″E	494.1	
15	JAU_2014	Dhari	21°19′28.3″N	1271.5	Gujarat
			71°01′08.5″E		
16	KHS_2013	Khas	22°13′17.5″N	2067.9	
17	KHS_2014	Khas	71°44′19.0″E		
18	MDS_2013	Modasa	23°26′57.9″N	1773.2	
19	MDS_2014	Modasa	73°17′54.7″E		

interaction effects of location (L), genotype (G) and genotype × location (G × L), respectively, using restricted maximum likelihood estimation (REML) procedure of GenStat 17 edition for windows (VSN International, Hemel Hempstead, UK, 2015) considering genotype and location as random. Individual location residuals were modeled into combined analysis; then, estimated variance components for all the traits were calculated (Table 3). Best linear unbiased predictors (BLUP's) for genotypes across locations were also estimated. Site regression model (SREG, commonly known as GGE Biplot) was used to visualize G × L pattern to understand interrelationships among various test locations and genotype evaluation.

The site regression model was:

$$Y_{ij} = \mu + \beta_j + \sum_{k=1}^{K} \lambda_k \delta_{ik} \beta_{jk} + \varepsilon_{ij}$$

where Y_{ij} is the mean yield of ith genotype in jth location, μ is the overall mean, δ_i is the genotypic effect, β_j is the location effect, λ_k is the singular value for IPCA axis k: δ_{ik} is the genotype eigenvector value for IPCA axis n, β_{jk} is the location eigenvector value for IPCA axis k, and ε_{ij} is the residual error assumed to be normally and independently distributed (0, σ^2/r), σ^2 is the pooled error variance, and r is the number of replicates. In the SREG model, the main

effects of genotypes (G) plus the G × L are absorbed into the multiplicative component. The GGE biplot graphically represents G and G × L effect present in the multi-location trial scaled to environment centered data. GGE biplots were in (1) genotype evaluation, stable genotype(s) determination across all locations and (2) location assessment that explains discriminative power among genotypes in target locations. The data from the biomass composition and TEY were analyzed in GenStat 14.1.

Results

Combined analysis of variance indicated that G, L and interaction effect G × L were showing highly significant (Prob > Z) differences among the high biomass sorghum genotypes tested (Table 3). The high biomass sorghum yields were significantly influenced by the test location, in fresh biomass yield the first two principal components PC1 and PC2 explained 38.9% variation (25.5 and 13.4%, respectively) (Fig. 2), whereas in dry biomass yield the first two principal components PC1 and PC2 explained 40.5% variation (27.3 and 13.1%, respectively) (Fig. 3). The two principal components PC1 and PC2 explained total variation of 82.9% (76.5 and 6.3%, respectively) in grain yield biplot (Fig. 4); although

Table 3 Estimated variance components for the various traits recorded from the 2 year multi-location trial in rainy season

| Random term	DFL	PHT_M	STEM GIRTH3	STEM GIRTH10	Inter nodes number	FSY	DSY	GY
	Z-value at 5%							
Loc	2.9** (55.9)	2.8** (40.0)	2.9** (92.4)	3.0** (92.6)	2.8** (47.9)	2.9** (69.4)	2.9** (57.9)	2.7** (74.9)
Geno	4.7** (23.4)	4.5** (21.6)	2.9** (0.9)	3.4** (1.2)	4.2** (15.6)	2.5** (2.7)	2.6** (4.1)	4.2** (12.0)
Loc × Geno	13.9** (20.6)	14.9** (38.2)	15.0** (6.6)	13.6** (6.0)	14.7** (36.4)	15.6** (27.8)	15.1** (37.9)	11.4** (13.0)

Values in parenthesis are component variance (%)

**Denotes significant difference ($P < 0.01$)

Loc location, *Rep* replication, *Geno* genotype, *DFL* days to 50% flowering, *PHT_M* plant height at maturity in meters, *STEM GIRTH 3* stem girth at 3rd internode (mm), *STEM GIRTH 10* stem girth at 10th internode (mm), *FSY* fresh biomass yield (t ha^{-1}), *DSY* dry biomass yield (t ha^{-1}), *GY* grain yield (t ha^{-1})
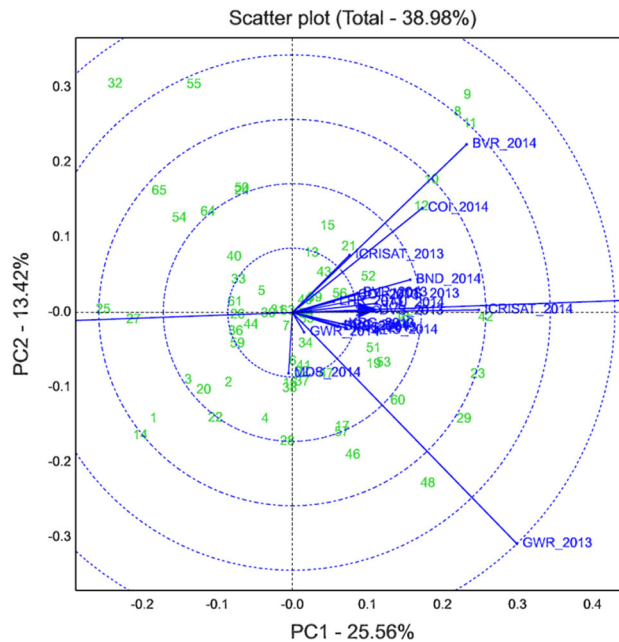
Fig. 2 The discriminating ability and representativeness the test environments for fresh biomass yield
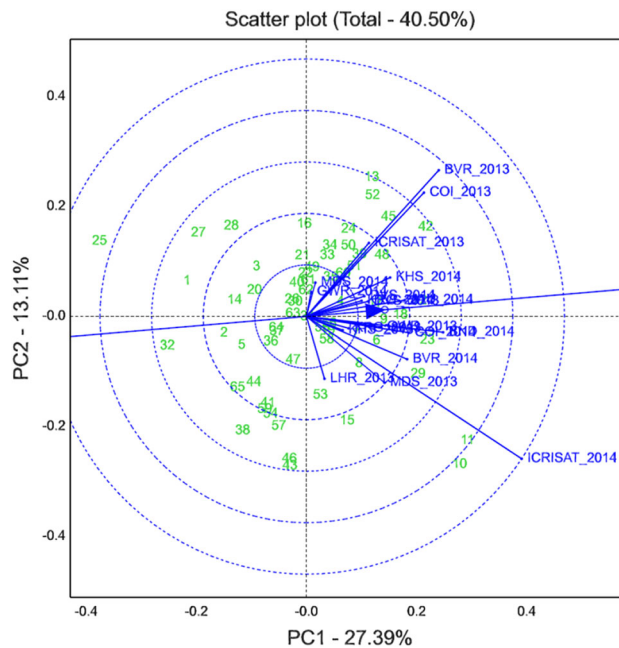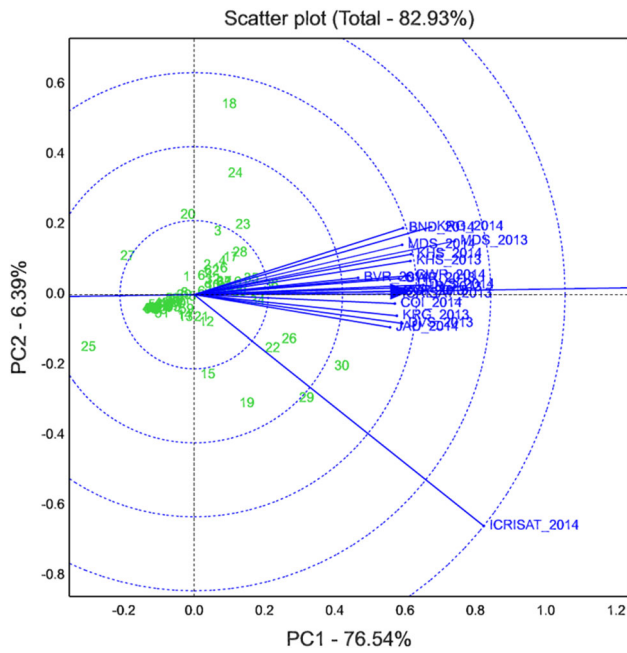

Fig. 3 The discriminating ability and representativeness of the test environments for dry biomass yield
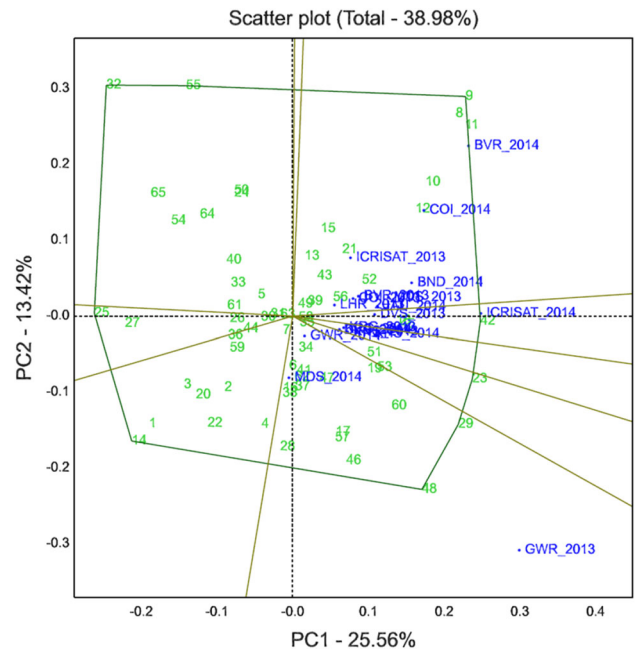
the percent variation unexplained for the fresh and dry biomass yield biplots is 61.0 and 59.5%.

Evaluation of the Environment Based on GGE

To visualize the relationship between the locations, lines drawn to connect the test locations to the origin of the

Fig. 4 The discriminating ability and representativeness the test environments for grain yield
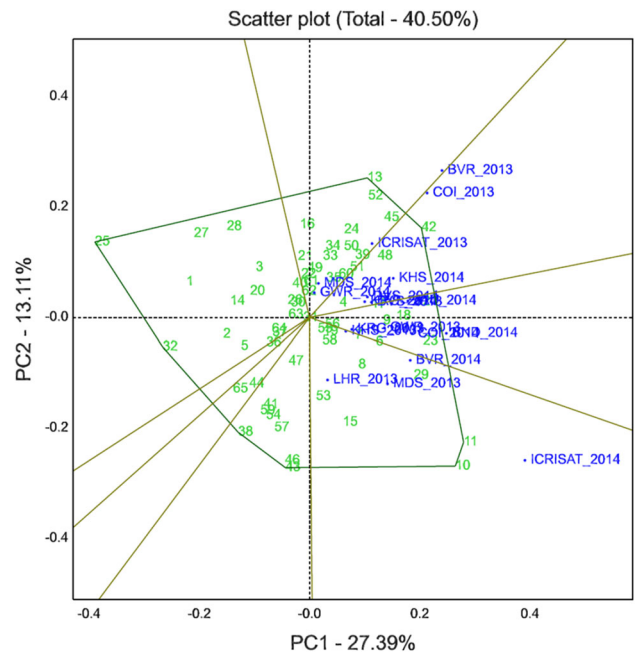

Fig. 5 The which-won-where view of the GGE biplot for fresh biomass yield

biplot (known as environmental vectors) are used. The cosine of angle between two environmental vectors explains the correlation between two locations (Yan et al. 2000; Yan and Tinker 2006). The test environments like Gwalior (GWR) in 2013, Coimbatore (COI) and Bhavanisagar (BVR) in 2014 were most discriminating (long environment vectors), while Modasa (MDS) in 2014 and Lahar (LHR) in 2013 were least discriminating environments (short environment vector) for fresh biomass yield (Fig. 2). The environments like COI in 2013 Khas (KHS) and Bhind (BND) in 2014 were representative but not discriminating for dry biomass yield where as ICRISAT in 2014, BVR and COI in 2013 were highly discriminating and GWR in 2014 was the least discriminating environment. The environments with specific adaptation for dry biomass yield are ICRISAT in 2014, BVR and COI in 2013, based on the length of their vectors (Fig. 3). In the grain yield GGE biplot, the location which is discriminating the test entries was ICRISAT in 2014 (Fig. 4). The environments in the grain yield biplot form a single cluster and hence show positive correlation with little GL interaction across the test locations.

Genotypic Performance

The performance of genotypes 8, 9, 10, 11 and 12 in year 2014 at BVR and COI and 48 in GWR was good, and these were adaptable genotypes for fresh biomass yield, in these discriminative locations. Genotypes 42, 52, 56, 39, 49, 25,

27, 26, 23 and 29 were stable across the locations. However, 42 was ideal because of highest fresh biomass yield. On the other hand, 25 and 27 were also highly stable but having lowest yield (Fig. 5). Similarly in dry biomass yield, genotypes 45, 52, 13, 42 were adaptable and high yielding genotypes in 2013 at BVR, COI and ICRISAT

Fig. 6 The which-won-where view of the GGE biplot for dry biomass yield
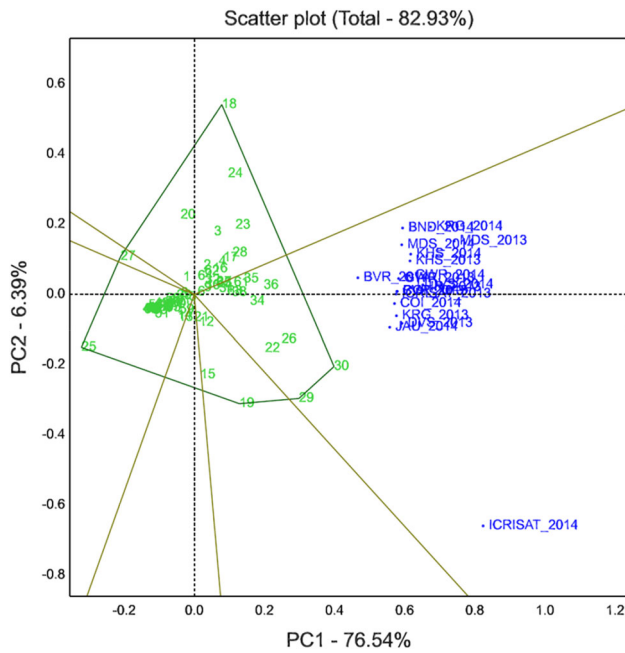
Fig. 7 The which-won-where view of the GGE biplot for grain yield

locations, whereas 25 and 32 were low yielders. Genotypes 10 and 11 were adaptable and high yielding at ICRISAT in 2014, and genotypes 9, 18, 32, 23 and 64 were highly stable across the locations (Fig. 6). In the GGE biplot for grain yield, genotypes 29 and 30 are high yielding and 18, 27 and 25 were low yielders in ICRISAT in 2014 (Fig. 7).

The fresh biomass yield biplot (Fig. 5) consists of five winner genotypes located on vertices of the polygon (genotype numbers: 9, 8, 11, 10 and 12), with one winner genotype (genotype number: 42) which was highly stable and high yielding across all locations in 2 years, and across GWR in 2013 and 2014 and MDS in 2014 the genotype numbers 48 and 29 are the winner genotypes. Similarly in the case of dry biomass yield biplot (Fig. 6) in 2013 at BVR, ICRISAT and COI, four winner genotypes (genotype numbers: 13, 52, 45 and 42) were identified, the 15 locations have four winner genotypes (6, 9, 18, and 23), the one location (ICRISAT in 2014) has 11 and 10 as the winner genotypes. The grain yield biplot (Fig. 7) has genotype numbers 34, 35, 36 and 38 as wining genotypes with 18 locations, and the one location (ICRISAT in 2014) has the genotype numbers 30 and 29 as the winner genotypes.

Theoretical Ethanol Yield (TEY)

The dry biomass yield for the selected top 20 genotypes from MLT was ranged from 11.9 to 19.2 t ha^{-1}. Although the dry biomass recorded has marginal differences for the genotypes 11 (18.9 t ha^{-1}) and 12 (19.2 t ha^{-1}), the major

Table 4 The dry stalk yield and predicted theoretical ethanol yield (TEY) for the top 20 lines based on the performance in MLTs conducted across test centers

Sl. no.	Genotype	Dry stalk yield[a] t ha^{-1}	TEY[a] (L ha^{-1})
1	ICSV25333	19.2	5494.1
2	IS13762	19.1	5391.7
3	CSH22SS	19.0	4690.5
4	IS25302	18.9	5545.0
5	IS25301	18.6	5287.7
6	IS27246	18.2	5252.1
7	IS16529	18.2	5321.2
8	DHBM-2	18.1	4848.5
9	ICSSH28	18.1	4629.9
10	IS17349	17.9	5199.8
11	IS17248	17.8	5242.8
12	IS25298	17.8	4652.8
13	IS22879	17.7	5233.0
14	DHBM-1	17.6	4425.5
15	ICSV93046	17.3	4401.4
16	IS22868	17.2	5532.2
17	Gird8	17.1	4628.8
18	IS25234	17.1	4775.5
19	IS13526	17.0	5253.2
20	IS23101	17.0	4833.9
21	SSG 59-3	11.9	3423.9

[a]Over all mean of all MLT locations

ethanol yield attributing trait (Table 4), the ethanol yield was more in 11 (5545 L ha^{-1}) followed by 51 (5532.2 L ha^{-1}) and 12 (5494.1 L ha^{-1}).

Discussion

One of the key focuses of the Indian sorghum breeding projects is to improve fodder quantity and quality in rainfed areas (Blümmel et al. 2003). The percent variance explained by the environment component is high, indicating that its influences on the biomass yield of genotypes are higher than the genotypic differences (Vange and Obi 2006; Reddy et al. 2014). Also, the percent variance explained by interaction effects of the G × E is higher than the genotypic variance (Yan and Hunt 2001) (Table 3). The multi-environment testing of genotypes to assess G × E interactions through genotypic yield stability plays an important role in either selecting widely adapted or specifically adapted genotypes to a particular test location. Therefore, studying yield performances, yield patterns and G × L of high biomass sorghum genotypes in the rainfed tropics of India is of cardinal importance for the identification of ideal genotypes for ideal test locations. The MLT

data may vary in the ranking of genotypes for yield traits across locations (Matus-Cadiz et al. 2003; Kaya et al. 2006; Gupta et al. 2013 due to biophysical and environmental interactions (Lin and Binns 1988). The test locations for MLT were chosen based on the representativeness in the rainfed tropics, as well as the potential areas where sorghum is grown extensively for fodder purpose. The targeted environments are mostly marginal land and encounter severe abiotic stress conditions like drought (Madhya Pradesh, Tamil Nadu and Telangana), salinity (Gujarat and Tamil Nadu) and water logging or flooding in Gujarat (Fig. 1). The test entries of high biomass sorghum showed differential performance for the fresh biomass, dry biomass and grain yield across all the testing locations are mainly due to the presence of genotypic variation, environmental effect as well as G × L interaction. High environmental variation indicates that the heritability of the observed variation is relatively low and improvement for biomass yield (fresh and dry) may not be proportional to the observed phenotypic variation. The high control of G × L interaction over the phenotypic variation further complicates selection for biomass genetic improvement as the phenotype will no longer be good predictor.

Soil and weather form the two main elements of an environment or test location influencing the performance of a genotype (Lin and Binns 1988). The soil element is generally persistent and may be regarded as fixed; and the weather element has a predictable component represented by the general climatic zone, and unpredictable component contributed by year-to-year variation (Yan et al. 2000; Dehghani et al. 2006, 2008; Sabaghnia et al. 2008). The presence of no close association between GWR and ICRISAT in 2013 with BVR in 2014 indicated that the effect of biophysical factors and climatic conditions plays a non-trivial role. The test location shows no similarities in performance for a single genotype tested and rather behave as individual locations. This is mainly attributed to the variable monsoon in these states, as the test locations in Madhya Pradesh had not received rainfall after sowing (annual rainfall in 2013 and 2014 in mm is 957.9 and 668.7, respectively) and in Gujarat the tests locations were over flooded due to heavy rains received (annual rainfall in 2013 and 2014 in mm is 2067.9 and 1773, respectively). The test locations two from each state were selected; hence, the non-discriminating test locations from Madhya Pradesh, Gujarat, Telangana and Tamil Nadu can be culled out. If two test environments are closely correlated consistently across years, one of them can be dropped without loss of much information about the genotypes (Yan and Tinker 2006).

Thus, GWR (Madhya Pradesh—Northern India) is different from that of BVR (Tamil Nadu) and ICRISAT (Telangana—Southern India), as they are present in different latitudes and altitudes. The high crossover GE,

order and performance of genotypes, varies according to the geographical conditions of testing environment, as the breeding and testing locations vary widely. These mega environment grouping is very explicit in the grain yield where only the ICRISAT location (Telangana) forms a mega environment and rest of the 18 locations forms a single cluster.

The variability for data on fresh biomass and dry biomass yield is significant. The average yield for fresh biomass across all the location is 34.5 t ha^{-1}, whereas the yield of genotype 42 (IS 13762—42.1 t ha^{-1}) is above the average yield; therefore, the genotype IS 13762 is stable and best performer across all the location in a combined analysis across 2 years. In 2013, at ICRISAT the genotype 21 (DHBM 5—26.4 t ha^{-1}) has an average fresh biomass yield recorded above the average of location (25.3 t ha^{-1}). Similarly in 2013, at LHR, the genotype 56 (IS 25234—57.4 t ha^{-1}) has recorded above the average fresh biomass yield of the location (49.6 t ha^{-1}). The genotype 34 (CSH 13—60.7 t ha^{-1}) has higher fresh biomass yield above the average fresh biomass yield in the location GWR (54.9 t ha^{-1}). The genotypes 8 (IS 18542—87.0 t ha^{-1}), 9 (IS 25298—82.4 t ha^{-1}) and 11 (IS 25302—78.1 t ha^{-1}) have higher than the average yield of the location for fresh biomass (49.6 t ha^{-1}) in BVR and COI in 2013; the genotypes 10 (IS 25301—47.8 t ha^{-1}) and 12 (ICSV 25333—66.6 t ha^{-1}) have above than the average yield (42.1 t ha^{-1}). The performance of these lines is not stable across all the locations, but these are best performers in the individual location, as the fresh biomass yield recorded is above average in these locations and thus are stable in these locations only (Supplementary material).

Furthermore, for dry biomass yield, the genotypes 18 (DHBM 2—18.4 t ha^{-1}), 23 (CSH 22SS—19.0 t ha^{-1}), 9 (IS 25298—17.7 t ha^{-1}), 32 (Chohatia—11.6 t ha^{-1}) and 63 (MP III—15.6 t ha^{-1}) for dry biomass yield were stable across all the locations (16.2 t ha^{-1}). The average dry biomass yield across all the location is 16.2 t ha^{-1}; thus, the lines CSH 22SS, DHBM 2, IS 25298 and MP III are best performers, but Chohatia is lower than the average yield. In 2013, ICRISAT the genotypes 39 (IS 13526—13.5 t ha^{-1}) and 48 (IS 17349—15.4 t ha^{-1}) are stable performers but has recorded lower than the average yield (19.7 t ha^{-1}), thus are low yielders. Likewise, in ICRISAT during 2014, the genotypes 11 (IS 25302—42.1 t ha^{-1}) and 10 (IS 25301—45.0 t ha^{-1}) have recorded higher than the average yield (25.5 t ha^{-1}), thus stable and best performers. The locations BVR and COI in 2013 have 13 (IS 27264—15.5 and 13.1 t ha^{-1}, respectively) and 52 (IS 22879—16.0 and 11.4 t ha^{-1}, respectively) as stable performers and have recorded higher than the average location yield (8.4 and 7.1 t ha^{-1}, respectively); thus, these lines are both stable and best performers in these locations. The genotypes 8 (IS

18542—19.0 t ha^{-1}) and 29 (ICSSH 28—19.5 t ha^{-1}) are stable and best as the average yield is higher than the location average yield (18.7 t ha^{-1}) in LHR, in 2013. The test location shows no similarities in performance for a single genotype tested and rather behave as individual locations. This is mainly attributed to the variable monsoon in these states, as the test locations in Madhya Pradesh had not received rainfall after sowing (annual rainfall in 2013 and 2014 in mm is 957.9 and 668.7, respectively) and in Gujarat the tests locations were over flooded due to heavy rains received (annual rainfall in 2013 and 2014 in mm is 2067.9 and 1773, respectively). The test locations two from each state were selected; hence, the non-discriminating test locations from Madhya Pradesh, Gujarat, Telangana and Tamil Nadu can be culled out. If two test environments are closely correlated consistently across years, one of them can be dropped without loss of much information about the genotypes (Comstock and Moll 1963). Visualization of "which-won-where" pattern of multi-environment yield trail (MEYT) data is necessary for studying the possible existence of different mega environments in the target environment (Lin and Binns 1988; Dehghani et al. 2008; Sabaghnia et al. 2008). The presence of close association between the test locations suggested that the same information about the genotypes could be obtained from fewer test environments, and hence, the potential to reduce evaluation costs. For fresh biomass yield and dry biomass yield the Bhavanisagar and Coimbatore locations shown the close association between them in terms of their performances, so one of the locations can be discarded. Hence, reiteration of test and evaluation of similar or different kind of genotypes will define the further the exactitude of the ideal environments in these states. Trials including new and common genotypes provide reliable information for future selections of test locations, even if the test is conducted for 1 year (Gupta et al. 2013) Decision to divide breeding locations into mega environments does not solely depend on the biological and statistical analyses of GL. Having a separate breeding program for each of the mega environments demands more logistics and research staff. In addition to the challenge to develop cultivars for different mega environments, the logistics for seed multiplication, distribution besides precise experimentation are crucial things to be considered before implementing specific adaptation breeding. Therefore, the pros and cons of breeding for specific adaptation need to be considered before embarking on it.

The biomass composition of IS 25302 reveals numerically higher cellulose and hemicellulose content than ICSV 25333, though, the dry biomass recorded has marginal differences for IS 25302 (18.9 t ha^{-1}) and ICSV 25333 (19.2 t ha^{-1}), the major ethanol yield attributing trait, a slight variation in biomass composition will affect the prediction of TEY in terms of dry biomass yield, cellulose and hemicellulose content (Cotton et al. 2013). These results are derived from the marginal lands of the test locations chosen and under lean period of drought, which shows remarkable performance in terms of yield and pose a viable option for establishing an economically viable ethanol production plant in these areas.

Conclusion

Sorghum is widely cultivated in Telangana, Madhya Pradesh, Gujarat and Tamil Nadu for use as a feed to the dairy animals. These areas are categorized under semiarid and rainfed tropic, to which sorghum is very well adapted. Although, with the development of new genotypes it is essential to evaluate the performance of these across locations and seasons for expanding the selection criteria, not only based on the yield but also the GGE interactions. Energy sorghum has dual advantage to the farmers in these areas: one is by providing the massive quantity of biomass owing to the long vegetative phase which meets the farmers fodder needs, and other is providing option to sell the surplus biomass for lignocellulosic conversion to yield biofuel. The crop in additional also answers the long debate of food versus fuel by providing suboptimal grain yield of 1.5–3.5 t ha^{-1} to the farmers. The test locations two from each state were selected; hence, the non-discriminating test locations from Madhya Pradesh, Gujarat, Telangana and Tamil Nadu can be culled out. If two test environments are closely correlated consistently across years, one of them can be dropped without loss of much information about the genotypes.

The entries like IS 16529, IS 22879, IS 27246, IS 13762, IS 25301 and ICSV 25333 were best suited for Tamil Nadu and Telangana, ICSSH 28, IS 17349 and ICSV 25333 were best suited for Madhya Pradesh, Gujarat and Telangana in terms of fresh biomass yield and dry biomass yield. The top 10 entries which are stable and showing best performance in terms of fresh biomass yield, dry biomass yield and grain yield were ICSV 25333, IS 13762, CSH22SS, IS 25302, IS 25301, IS 27246, IS 16529, DHBM2, ICSSH 28 and IS 17349. Thus, the lines like ICSV 25333, IS 25302, IS 22868, IS 13762 and IS 16529 are stable performers both in terms of biomass accumulation and economically viable for ethanol production.

Blümmel M, ILRI, in analyzing the composition of biomass samples in NIRS.

Compliance with Ethical Standards

Conflict of interest All the authors declare that there is no conflict of interest in publishing Identification of ideal locations and stable high biomass sorghum genotypes in semiarid tropics in Sugar Tech.

References

Antonopoulou, G., H.N. Gavala, I.V. Skiadas, K. Angelopoulos, and G. Lyberatos. 2008. Biofuels generation from sweet sorghum: Fermentative hydrogen production and anaerobic digestion of the remaining biomass. *Bioresource Technology* 99(1): 110–119.

Berndes, G., M. Hoogwijk, and R. van den Broek. 2003. The contribution of biomass in the future global energy supply: A review of 17 studies. *Biomass and Bioenergy* 25(1): 1–28.

Bidinger, F.R., and M. Blümmel. 2007. Effects of ruminant nutritional quality of pearl millet [*Pennisetum glaucum* (L) R. Br.] stover. 1. Effects of management alternatives on stover quality and productivity. *Field Crops Research* 103(2): 129–138.

Blanche, S.B., and G.O. Myers. 2006. Identifying discriminating locations for cultivar selection in Louisiana. *Crop Science* 46(2): 946–949.

Blümmel, M., F.R. Bidinger, and C.T. Hash. 2007. Management and cultivar effect on ruminant nutritional quality of pearl millet [*Pennisetum glaucum* (L) R. Br.] stover. Effects of cultivar choice on stover quality and productivity. *Field Crops Research* 103(2): 119–128.

Blümmel, M., S. Deshpande, J. Kholova, and V. Vadez. 2015. Introgression of staygreen QLT's for concomitant improvement of food and fodder traits in *Sorghum bicolor*. *Field Crops Research* 180: 228–237.

Blümmel, M., E. Zerbini, B.V.S. Reddy, C.T. Hash, F. Bidinger, and A.A. Khan. 2003. Improving the production and utilization of sorghum and pearl millet as livestock feed: Progress towards dual-purpose genotypes. *Field Crops Research* 84(1): 143–158.

Brown, K.D., M.E. Sorrels, and W.R. Coffman. 1983. A method for classification and evaluation of testing environments. *Crop Science* 23: 889–893. https://doi.org/10.2135/cropsci1983.0011183X002300050018x.

Buxton, D.R., I.C. Anderson, and A. Hallam. 1999. Performance of sweet and forage sorghum grown continuously, double-cropped with winter rye, or in rotation with soybean and maize. *Agronomy Journal* 91(1): 93–101.

Comstock, R.E., and R.H. Moll. 1963. Genotype–environment interactions. In *Statistical genetics and plant breeding*, ed. W.D. Hanson and H.F. Robinson, 164–196. Washington: National Academy of Sciences-National Research Council.

Cotton, J., G. Burow, V. Acosta-Martinez, and J. Moore-Kucera. 2013. Biomass and cellulosic ethanol production of forage sorghum under limited water conditions. *BioEnergy Research* 6(2): 711–718.

Dehghani, H., A. Ebadi, and A. Yousefi. 2006. Biplot analysis of genotype by environment interaction for barley yield in Iran. *Agronomy Journal* 98: 388–393.

Dehghani, H., H. Omidi, and N. Sabaghnia. 2008. Graphic analysis of trait relations of rapeseed using the biplot method. *Agronomy Journal* 100: 1443–1449.

Dolciotti, I., S. Mambelli, S. Grandi, and G. Venturi. 1998. Comparison of two sorghum genotypes for sugar and fiber production. *Industrial Crops and Products* 7(2): 265–272.

Ejeta, G., and J.E. Knoll. 2007. Marker-assisted selection in sorghum. In *Genomics-assisted crop improvement*, ed. R.K. Varshney and R. Tuberosa, 187–205. Dordrecht: Springer.

Gill, John R., Payne S. Burks, Scott A. Staggenborg, Gary N. Odvody, Ron W. Heiniger, Bisoondat Macoon, Ken J. Moore, Michael Barrett William, and L. Rooney. 2014. Yield Results and Stability Analysis from the Sorghum Regional Biomass Feedstock Trial. *BioEnergy Research* 3: 1026–1034.

Gupta, S.K., A. Rathore, O.P. Yadav, K.N. Rai, I.S. Khairwal, B.S. Rajpurohit, and R.R. Das. 2013. Identifying mega-environments and essential test locations for pearl millet cultivar selection in India. *Crop Science* 53(6): 2444–2453.

Institution of Japan Energy, ed. 2006. *Biomass handbook*. Trans. Z.Z. Hua and Z.P. Shi, 166–167, 172. Beijing: Chemistry Industry Press **(in Chinese)**.

Kaya, Y., M. Akcura, and S. Taner. 2006. GGE-biplot analysis of multi-environment yield trials in bread wheat. *Turkish Journal of Agriculture and Forestry* 30: 325–337.

Lin, C.S., and M.R. Binns. 1988. A method of analyzing cultivar 9 location 9 year experiments: A new stability parameter. *Theoretical and Applied Genetics* 76: 425–430.

Mamma, D., P. Christakopoulos, D. Koullas, D. Kekos, B.J. Macris, and E. Koukios. 1995. An alternative approach to the bioconversion of sweet sorghum carbohydrates to ethanol. *Biomass and Bioenergy* 8(2): 99–103.

Matheson, A.C., and C.A. Raymond. 1986. A review of provenance × environment interaction. Its practical importance and use with particular reference to the tropics. *Commonwealth Forestry Review* 65: 283–302.

Matus-Cadiz, M.A., P. Hucl, C.E. Perron, and R.T. Tyler. 2003. Genotype × environment interaction for grain color in hard white spring wheat. *Crop Science* 43(1): 219–226.

Meki, M.N., J.L. Snider, J.R. Kiniry, R.L. Raper, and A.C. Rocateli. 2013. Energy sorghum biomass harvest thresholds and tillage effects on soil organic carbon and bulk density. *Industrial Crops and Products* 43: 172–182.

Nagaiah, D., P. Srinivasa Rao, and R.S. Prakasham. 2012. High biomass sorghum as a potential raw material for biohydrogen production: A preliminary evaluation. *Current Trends in Biotechnology and Pharmacy* 6(2): 183–189.

Olson, S.N., K. Ritter, J. Medley, T. Wilson, W.L. Rooney, and J.E. Mullet. 2013. Energy sorghum hybrids: Functional dynamics of high nitrogen use efficiency. *Biomass and Bioenergy* 56: 307–316.

Olson, Sara N., Kimberley Ritter, William Rooney, Armen Kemanian, Bruce A. McCarl, Yuquan Zhang, Susan Hall, Dan Packer, and John Mullet. 2012. High biomass yield energy sorghum: Developing a genetic model for C4 grass bioenergy crops. *Biofuels, Bioproducts and Biorefining* 6: 640–655.

Packer, D.J., and W.L. Rooney. 2014. High-parent heterosis for biomass yield in photoperiod-sensitive sorghum hybrids. *Field Crops Research* 167: 153–158.

Peterson, C.J., and W.H. Pfeiffer. 1989. International winter wheat evaluation: Relationships among test sites based on cultivar performance. *Crop Science* 29: 276–282. https://doi.org/10.2135/cropsci1989.0011183X002900020008x.

Pham, H.N., and M.S. Kang. 1988. Interrelationships among and repeatability of several stability statistics estimated from international maize trials. *Crop Science* 28(6): 925–928.

Rakshit, S., K.N. Ganapathy, S.S. Gomashe, A. Rathore, R.B. Ghorade, M.N. Kumar, et al. 2012. GGE biplot analysis to evaluate genotype, environment and their interactions in sorghum multi-location data. *Euphytica* 185(3): 465–479.

Rattunde, H.F., E. Zerbini, S. Chandra, and D.J. Flower. 2001. Stover quality of dual-purpose sorghums: Genetic and environmental sources of variation. *Field Crops Research* 71(1): 1–8.

Reddy, P.S., B.V.S. Reddy, and P.S. Rao. 2014. Genotype by sowing date interaction effects on sugar yield components in sweet sorghum (*Sorghum bicolor* L. Moench). *SABRAO Journal of Breeding and Genetics* 46(2): 241–255.

Rinne, M., S. Jaakkola, and P. Huhtanen. 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. *Animal Feed Science and Technology* 67(1): 1–7.

Sathya, A., K.S. Vinutha., P.S. Rao, and S. Gopalakrishnan. 2016. Cultivation of sweet sorghum on heavy metal contaminated soils by phytoremediation approach for production of bioethanol. In *Bioremediation and Bioeconomy*, ed. M.N.V. Prasad, 271–286. Amsterdam: Elsevier. ISBN 9780128028308.

Sabaghnia, N., H. Dehghani, and S.H. Sabaghpour. 2008. Graphic analysis of genotype by environment interaction for lentil yield in Iran. *Agronomy Journal* 100: 760–764.

Srinivasa Rao, P., P.S. Reddy, A. Rathore, B.V. Reddy, and Panwar Sanjeev. 2011. Application GGE biplot and AMMI model to evaluate sweet sorghum (*Sorghum bicolor*) hybrids for genotype × environment interaction and seasonal adaptation. *Indian Journal of Agricultural Sciences* 81(5): 438–444.

Thomason, W.E., and S.B. Phillips. 2006. Methods to evaluate wheat cultivar testing environments and improve cultivar selection protocols. *Field Crops Research* 99(2): 87–95.

Vange, T., and I.U. Obi. 2006. Effect of planting date on some agronomic traits and grain yield of upland rice varieties at Makurdi, Benue state, Nigeria. *Journal of Sustainable Development in Agriculture and Environment* 2: 1–9.

Yan, W., and L.A. Hunt. 2001. Interpretation of genotype × environment interaction for winter wheat yield in Ontario. *Crop Science* 41(1): 19–25.

Yan, W., and I. Rajcan. 2002. Biplot analysis of test sites and trait relations of soybean in Ontario. *Crop Science* 42: 11–20.

Yan, W., and M.S. Kang. 2003. *GGE biplot analysis: A graphical tool for breeders, geneticists and agronomists*. 1st ed. Boca Raton, FL: CRC Press. ISBN-13: 9781420040371.

Yan, W., and N.A. Tinker. 2006. Biplot analysis of multi-environment trial data: Principles and applications. *Canadian Journal of Plant Science* 86: 623–645.

Yan, W., L.A. Hunt, Q. Sheng, and Z. Szlavnics. 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. *Crop Science* 40: 597–605.

Zhao, Y.L., A. Dolat, Y. Steinberger, X. Wang, A. Osman, and G.H. Xie. 2009. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. *Field Crops Research* 111(1): 55–64.