Range Mgmt. & Agroforestry 40 (1) : 59-66, 2019
ISSN 0971-2070

Selection of sorghum [*Sorghum bicolor* (L.) Moench] genotypes for drought tolerance using physiological characterization

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Received: 8th August, 2018 Accepted: 28th April, 2019

Abstract
Sorghum [*Sorghum bicolor* (L.) (Moench] genotypes are potential reservoir for genetic improvement towards various biotic and abiotic stresses. The genotypic differences were investigated among the thirty genotypes including a tolerant (B35) and susceptible (Co26) cultivar to moisture stress tolerance in field condition. In our study, the genetic variability parameters such as, phenotypic and genotypic coefficient of variation was higher for epicuticular wax (98.01) and leaf rolling (72.62), respectively. Likewise, higher heritability and expected genetic advance as per cent mean was reported for net photosynthetic rate (96.60) and leaf rolling (143.59), respectively. Correlation coefficient analysis found that most of the putative traits had significant association with grain yield *viz.,* stay green score (r=0.80**), transpiration rate (0.67**), chlorophyll content (r=0.66**), relative water content (r=0.65**), early ground cover (0.58**), net photosynthetic rate (0.54**), root length (0.38*), epicuticular wax (0.30) and leaf rolling (0.10). Its genetic diversity was assessed through putative physiological trait expressions based on mean performance resulted in four clusters *viz.,* I, II, III and IV consisting of 5, 12, 4 and 9 genotypes, respectively. Cluster I comprised superior genotypes along with tolerant cultivar. Further, principle component analysis revealed three components each explained by 47.45%, 15.83% and 12.18%, respectively of total variation based on putative physiological traits. Accordingly, biplot analysis revealed that four genotypes *viz.*, DRT1026, ICSR24001, DRT1030 and DRT1019 showed superior performance for drought tolerance. The pattern of physiological responses and genetic diversity offers further opportunity for genetic resource conservation and utilization for genetic improvement of sorghum for drought tolerance.

Keywords: Drought tolerance, Genetic resources, Physiology, Sorghum

Introduction
Drought or moisture stress is one of the most widespread environmental stresses and limits crop production (Arora *et al.,* 2002; Adugna, 2007). It affects germination and different growth stages of crop plants through impaired physiological and metabolic processes (Khayatnezhad *et al.,* 2010). Sorghum is an important forage and food crop in arid and semiarid regions of India (Deb *et al*., 2014; Rajarajan and Ganesamurthy, 2014), In India sorghum mainly grown under rainfed condition in many states for food and fodder purpose (Motlhaodi *et al.,* 2016; Das and Patil, 2013). However, sorghum production under rainfed condition is severely affected by drought resulting in negative impact on yield (Ali *et al.,* 2011). Generally, drought response of sorghum is categorized into pre and post-flowering stages and (moisture) stress response of post-flowering sorghum is considered to be most important as it may reduce grain yield. Modern sorghum varieties contribute limited genetic potential towards improvement and development of new varieties with increased grain yield under drought stress condition. Thus genetic improvement for drought tolerance in association with stable grain yield of sorghum is considered as an essential effort. Despite numerous studies showing that morphological responses and marker-based analysis of sorghum to stress, very limited work has been undertaken to evaluate and understanding the various physiological responses of genotypes; particularly, photosynthesis and other related physiological perspective. In this scenario it is necessary to understand the genetic diversity in association with strong physiological responses to enhance sorghum production under drought environment. With this view, the present study was undertaken to evaluate sorghum genotypes for genetic variation, to assess genetic relationship among various putative traits under drought and to identify a most tolerant genotype using physiological indicator traits under moisture stress condition.

Materials and Methods

Plant materials: The plant materials were comprised of 30 diversified forage and grain sorghum genotypes (selected from first year study comprised of 100 genotypes) and provided by department of Plant Genetic Resources (PGR), Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The genotypes were improved lines and varieties originally collected from ICRISAT (International Crop Research Institute for Semi-Arid Tropics, Hyderabad, India) and IIMR (Indian Institute of Millets Research, Hyderabad, India) including tolerant and susceptible cultivars B35 and Co26, respectively from Tamil Nadu Agricultural University, Coimbatore, India (Table 1).

Table 1. Thirty sorghum genotypes used in this study along with their source

S. No.	Genotypes	Source
1	ICSR93001	ICRISAT, India
2	DRT1026	ICRISAT, India
3	ICSR24001	ICRISAT, India
4	DRT1030	ICRISAT, India
5	IS23399	ICRISAT, India
6	MS7735	Unknown
7	RS14432	ICRISAT, India
8	ICSV202	ICRISAT, India
9	KO5SS202	IIMR, India
10	KO5SS150	IIMR, India
11	KO5SS25	IIMR, India
12	KO5SS53	IIMR, India
13	KO5SS302	IIMR, India
14	KO5SS267	IIMR, India
15	KO5SS450	IIMR, India
16	KO5SS38	IIMR, India
17	KO5SS186	IIMR, India
18	KO3SS127	IIMR, India
19	B35 (T)*	ICRISAT, India
20	ICSV587	ICRISAT
21	DRT1019	ICRISAT, India
22	IS5005	ICRISAT, India
23	IS1130	ICRISAT, India
24	IS3552	ICRISAT, India
25	AS5160	Unknown
26	MS8444	Unknown
27	VS1565	Unknown
28	KO5SS244	IIMR, India
29	CO26 (S)*	TNAU, India
30	ICSV95022	ICRISAT, India

**T: Tolerant and S: Susceptible check*

Experimental condition and traits quantification: The field experiment was conducted at Tamil Nadu Agricultural University, Coimbatore, India during summer 2017. The experiment was laid out in randomized block design with two replications and standard agronomic practices were adopted. One set of treatment with normal irrigation from planting to maturity served as control. Another set of treatment as drought stress imposed by withholding irrigation at anthesis stage and continued till maturity. The data was recorded on five randomly selected plants from each plot for the physiological traits. The observations were taken on chlorophyll content (SPAD index) (CHY), relative water content (RWC), epicuticular wax (ECW), leaf rolling (LR), digital ground cover (DGC), transpiration rate (E), net photosynthetic rate (P_N), stay-green score (SGR) and grain yield per plant (GYP). The total leaf chlorophyll contents were measured with a Minolta chlorophyll meter SPAD-502. Relative water content (RWC) was calculated as (%) using the formula suggested by Barrs and Weatherly (1962). Epicuticular wax (mg/dm^2) was estimated by the method suggested by Ebercon *et al.* (1977). Visual leaf rolling was estimated as suggested by O'Toole and Moya (1978) used a scale of rolling from 1 to 5 (1 being only the evidence of rolling, while 5 the severest with leaf being a closed cylinder). Early ground cover (%) was measured based on the method suggested by Mullan and Reynolds (2010). Leaf gas-exchange variables *viz*. transpiration rate (mmol (H_2O) m^{-2}s^{-1}) and net photosynthetic rate (μmol (CO_2) m^{-2}s^{-1}) were measured from the fourth and fifth leaves from each genotype (Resende *et al.,* 2012) using an open gas exchange system with a 6 cm^2 clamp-on leaf cuvette (LI-6400, LICOR Inc., Lincoln, NE, USA). These measurements were accomplished on clear sunshine days between 10 am to 12 noon. The stay-green score was estimated visually on a plot basis on a scale of 1 to 5 based on the degree of leaf and plant senescence at physiological maturity under drought stress, by following the visual ratings of stay-green trait suggested by Wanous *et al.* (1991). Root length was measured by giving a deep dig near the base after watering uprooted plant and the maximum root length of the longest root was measured in centimeter under field condition. The grain yield per plant was estimated as weight of the dried and cleaned grains from a single plant and expressed in grams.

Statistical data analysis: The genetic variability parameters were performed with INDOSTAT version 8.5 (Hyderabad, India). Correlation co-efficient, cluster analysis based on unweighted paired group arithmetic mean (UPGMA) and principle component analysis were performed with XLSTAT 2017: Addinsoft, Paris, France (2017).

Results and Discussion

The mean performance of thirty sorghum genotypes for the biometrical and physiological traits (Table 2) under drought stress showed high variation among the genotypes as it was evident from the range of values.

The analysis of variance was significant for all the traits considered and justified the relative contributions of these traits to the variability (Table 3). These genotypes also significantly differed for drought tolerant indicator traits indicating a substantial influence of environment on these traits.

Table 2. Mean values of physiological stress indicator traits and grain yield parameters of thirty sorghum genotypes under drought condition

Genotypes	CHY	RWC	ECW	LR	DGC	E	P_N	SGR	RL	GYP
ICSR93001	57.90	53.02	0.52	2.00	72.89	5.30	15.57	2.00	35.00	37.10
ICSR24001	58.00	58.09	0.48	2.00	73.28	5.39	32.90	2.00	38.10	37.60
DRT1026	48.40	69.23	0.16	3.00	81.39	1.99	30.03	2.00	30.00	39.00
DRT1030	56.00	60.05	0.48	3.00	76.28	5.31	33.39	1.00	36.00	50.10
IS23399	45.80	51.00	0.36	3.00	59.74	3.50	20.13	5.00	17.00	23.90
MS7735	48.20	24.09	0.36	2.00	62.15	5.01	21.98	1.00	21.00	49.70
RS14432	40.30	47.26	0.48	0.00	45.91	3.04	16.69	2.00	30.00	34.20
ICSV202	50.90	36.90	0.48	2.00	40.21	3.25	22.45	1.00	30.30	40.00
KO5SS202	48.03	58.23	0.16	5.00	50.79	4.77	27.42	5.00	33.00	38.00
KO5SS150	45.40	37.14	0.56	1.00	57.13	4.33	21.42	2.00	32.10	40.50
KO5SS25	47.60	38.01	0.56	1.00	43.67	3.92	21.83	2.00	36.10	32.20
KO5SS53	48.50	66.11	0.48	1.00	52.77	4.89	21.92	2.00	38.10	43.00
KO5SS302	49.90	58.61	0.24	1.00	56.30	2.96	14.18	2.00	20.10	32.10
KO5SS267	40.90	49.28	0.72	0.00	59.01	3.25	20.09	2.00	25.10	35.20
KO5SS450	47.60	50.39	0.68	1.00	76.28	3.21	18.59	3.00	26.50	33.30
KO5SS38	46.00	54.59	0.96	2.00	61.28	3.35	15.27	2.00	30.50	37.70
KO5SS186	45.30	53.46	1.64	2.00	64.28	4.39	26.97	1.00	23.00	47.55
KO3SS127	50.10	30.00	1.20	2.00	59.93	4.59	22.80	3.00	20.50	28.80
B35	58.20	70.56	0.53	3.00	80.39	5.78	35.95	1.00	41.00	50.61
ICSV587	32.39	24.53	0.20	1.00	47.69	1.99	15.93	4.00	30.80	21.80
DRT1019	59.30	70.23	0.73	3.00	79.30	5.78	33.93	2.00	29.33	48.30
IS5005	43.20	34.35	0.48	1.00	40.50	4.27	21.88	4.00	20.50	33.40
IS1130	54.10	35.32	1.16	1.00	52.14	3.73	17.23	4.00	18.00	29.40
IS3552	28.10	20.51	0.13	1.00	36.21	1.98	13.78	4.00	30.00	23.20
AS5160	46.90	20.95	0.52	3.00	55.46	4.50	33.37	5.00	25.00	23.30
MS8444	44.40	26.94	0.12	1.00	40.09	3.16	25.38	5.00	40.50	26.70
VS1565	48.30	49.38	0.48	1.00	45.98	2.98	15.08	3.00	26.50	30.00
KO5SS244	46.90	49.30	1.20	1.00	49.58	3.60	20.90	5.00	24.00	32.30
CO26	34.10	23.51	0.10	4.00	57.92	3.08	18.00	5.00	22.00	20.40
ICSV95022	28.30	20.93	0.14	3.00	39.23	2.00	14.98	5.00	21.00	20.81
Mean	**47.47**	**39.05**	**0.73**	**2.03**	**57.09**	**3.82**	**22.26**	**2.82**	**28.83**	**35.57**

Table 3. Analysis of variance of ten putative physiological trait responses of sorghum genotypes under drought condition

Source of variation	df	Mean sum of squares									
		CHY	RWC	ECW	LR	DGC	E	P_N	SGR	RL	GYP
Replication	1	399.26	246.19	0.03	0.04	288.28	1.16	40.42	0.58	60.92	98.56
Treatments	29	228.07**	385.23**	0.64**	4.54**	350.18**	2.47**	85.13**	4.00**	115.12**	161.37**
Error	29	41.86	22.82	0.38	0.18	9.85	0.04	1.47	0.55	2.55	3.53

CHY: Chlorophyll content (SPAD index), RWC: Relative water content, ECW: Epicuticular wax, LR: Leaf rolling score, DGC: Digital ground cover, E: Transpiration rate, P_N: Net photosynthetic rate, SGR: Stay-green score, RL: Root length, GYP: Grain yield/plant ; **significant at p < 0.01

Genetic variability: Generally, drought tolerance is a complex trait due to high level of association between genotype and environment; genotypes differ in drought tolerance serve as important system for studying adaptive responses to drought in crop species (Cooper *et al.,* 2006). The genetic relationship and variability among the 30 sorghum genotypes were dissected into different components through putative physiological drought stress indicator parameters. The percent phenotypic (PCV) and genotypic (GCV) coefficient of variation for all the ten characters were estimated under drought condition (Table 4). All the thirty genotypes exhibited high level of genetic variation for most of the quantitative traits considered, which provides more opportunities for effective selection. The coefficient of variation coupled with heritability is useful in determining the heritable portion that could help ineffective selection (Burton, 1952). In the present study, all the drought indicator parameters studied showed intermediate to high level of GCV (20.32 to 72.62%), high estimates of heritability (24.85 to 96.60%) for the traits considered (except ECW) and high expected genetic advance (34.77 to 143.59%). These genetic variability estimates indicated high magnitude of variation present across the thirty genotypes for drought tolerance; also these characters were found to be under the control of additive gene action. Similarly, Falconer and Mackay (1996) reported that additive genetic variance measured by heritability and genetic advance of a population can effectively dissect out the potential for adaptive evolution to the defined environment. In our study, the results indicated that most of the physiological traits along with GYP had high estimate for GCV, heritability, and expected genetic advance reflecting additive genes controlling these trait and selection might be effective for drought tolerance improvement. Further this population could be used in breeding programs for developing improved sorghum cultivars with a broad genetic base (Bafeel, 2015).

Correlation coefficient: Correlation coefficient is an important biometrical tool for formulating the selection index, as it reveals the strength of relationship among the group of characters. In our study, most of the traits under consideration had significant association with grain yield and other physiological parameters under drought condition (Table 5). Among the traits SGR had strong association with GYP ($r = 0.80^{**}$). Similarly sorghum grain size was correlated with relative reduction rate of leaf senescence during grain filling as it doubled the grain size about 15 mg to 30 mg and had a potential to increase sorghum grain yield by improving both grain number and grain filling ability under drought condition (Borrell *et al.,*1999). Further CHY ($r=0.66^{**}$) and RWC ($r=0.65^{**}$) had strong association with grain yield, respectively. These results were in agreement with Getnet *et al.* (2015). They reported RWC ($r=0.86^{**}$) and CHY ($r=0.75^{**}$), had strong positive association with grain yield. Thus breeding lines possessing the stay-green trait tended to remain green for longer period as it maintained the integrity of chloroplast proteins such as LUCP2, OEC33 and Rubisco, thereby they had a potential to increase sorghum grain yield by improving both grain number and grain filling ability (Borrell *et al.*, 1999; 2001).

In our study, the gas exchange parameters such as E ($r=0.67^{**}$) and P_N ($r=0.54^{**}$) had significant positive association with GYP. Similarly Vadez *et al.* (2011) reported that in sorghum grain yield showed a highly significant positive correlation with Transpiration efficiency (TE) through effective E (R^2 = 0.60). The enhanced TE might benefit sorghum production through accumulated more biomass, and possibly higher grain

Table 4. Phenotypic (PCV) & genotypic (GCV) coefficient of variation, heritability (h^2) and expected genetic advance (GA%) for ten characters under drought stress condition

Characters	PCV	GCV	h^2	GA %
Chlorophyll content (SPAD) index	24.47	20.32	68.97	34.77
Relative water content	36.57	34.46	88.81	66.91
Epicuticular wax	98.01	48.86	24.85	50.18
Lear rolling score	75.66	72.62	92.12	143.59
Digital ground cover	23.49	22.84	94.52	45.75
Transpiration rate	29.40	28.87	96.44	58.41
Net photosynthetic rate	29.55	29.05	96.60	58.82
Stay-green score	53.45	46.44	75.47	83.11
Root length	26.59	26.01	95.66	52.41
Grain yield/plant	25.52	24.97	95.70	50.32

yield and might be a relevant trait in enhancing sorghum yield under water-limited conditions (Impa *et al.,* 2005; Xin *et al.,* 2009). Similarly in sorghum total biomass accumulation was a function of P_N and growth duration since, higher leaf photosynthetic rates was necessary for increased yields (Peng *et al.,* 1991). Chandra Babu *et al.* (1985) found significant and positive correlations between leaf photosynthetic rates during the post-anthesis period total dry matter production and pod yield for 20 genotypes of blackgram. However, high photosynthetic efficiency was achieved through low internal CO_2 concentration and high TE that was associated with high biomass/yield of sorghum lines (Xin *et al.,* 2009). Hence, understanding this putative physiological indicator parameter with grain yield could help in further genetic improvement of sorghum in respect with grain yield under drought condition.

In our study, DGC trait had significant positive association with GYP (r=0.58). Similarly Bellundagi *et al.* (2013) reported that wheat genotypes had significant positive association of DGC with GYP (r=0.66**). Early vigour is a physiological trait characterized by rapid development of leaf area considered an important trait response for drought tolerance in association with stable grain yield (Richards and Lukacs, 2002). This kind of association suggested the possibilities of improvement of this character through selection. The RL in our study, had significant association with GYP (r=0.38**) under drought condition. Similar results were obtained by Sinde *et al.* (2017) as *rabi* grain sorghum under drought condition. The RL significantly correlated with GYP (r=6.49**). Root responses are the major trait expression especially in low soil moisture availability as it uptakes water from the deeper soil by extension of root length. Also genotypes with higher root length tended to have high leaf water potential and delayed leaf death by maintaining favorable plant water status resulting in higher grain yield under water limited conditions (Ekanayake *et al.,* 1985). In our study, LR (0.10) and ECW (0.30) had less association with GYP. Similarly Rauf *et al.* (2015) reported that in maize the LR was correlated with GYP (r=-0.18). However, the strong correlation of LR and ECW with grain yield allows breeders to use these as effective selection criteria for drought resistance in plants (Hsiao *et al.,* 1984). Thus quantifying the association between LR and ECW with GYP will greatly improve grain yield through selection.

Genetic diversity: Agglomerative hierarchical clustering performed on the Euclidean distance matrix utilizing Ward's linkage method under drought condition had resulted in classifying 30 sorghum accessions into four clusters and the size varied from 4 to 12 (Fig 1). Cluster II consisted of maximum genotypes (12), followed by cluster IV (9), cluster I (5) and cluster II (4). Further the cluster II formed by four genotypes along with tolerant cultivar (B35) exhibited its drought tolerance due to higher expression of putative traits along with yield (Table 6). Similar pattern of clustering was reported on sorghum landraces based on morpho-physiological traits under post flowering drought by Abraha *et al.* (2015). Hence, in our study cluster I was more stable against drought without compromising considerable yield. Contrastingly, cluster III had four genotypes along with susceptible cultivar Co26 might be due to lower putative trait responses.

Table 5. Correlation coefficient of ten putative physiological traits under drought condition

Variables	RWC	ECW	LR	DGC	E	P_N	SGR	RL	GYP
CHY	0.65**	0.34	0.15	0.63**	0.76**	0.57**	0.50**	0.30	0.66**
RWC		0.19	0.14	0.65**	0.41*	0.41*	0.49**	0.36	0.65**
ECW			-0.23	0.20	0.31	0.09	0.25	-0.25	0.30
LR				0.35	0.25	0.47**	0.19	0.10	0.10
DGC					0.51**	0.55**	0.48**	0.18	0.58**
E						0.64**	0.39**	0.31	0.67**
P_N							0.24	0.40**	0.54**
SGR								0.33	0.80**
RL									0.38*

$^{*,\ **}$ *Significant at P<0.05 and P<0.01, respectively;*

CHY: Chlorophyll content (SPAD index), RWC: Relative water content, ECW: Epicuticular wax, LR: Leaf rolling score, DGC: Digital ground cover, E: Transpiration rate, P_N: Net photosynthetic rate, SGR: Stay-green score, RL: Root length, GYP: Grain yield/plant

Table 6. Cluster composition of thirty genotypes under different clusters

Cluster No.	Genotype compositions
I	ICSR24001 DRT1026 DRT1030 B35 DRT1019
II	ICSR93001 IS23399 RS14432 KO5SS202 KO5SS53 KO5SS302 KO5SS267 KO5SS450 KO5SS38 KO5SS186 VS1565 KO5SS244
III	ICSV587 IS3552 CO26 ICSV95022
IV	MS7735 ICSV202 KO5SS150 KO5SS25 KO3SS127 IS5005 IS1130 AS5160 MS8444

Table 7. First three principle components for the 30 sorghum genotypes

Traits	Estimated factor loadings genotypes under drought stress condition		
	PC1	PC2	PC3
Chlorophyll content (SPAD index	**0.74**	0.00	0.02
Relative water content	**0.59**	0.00	0.01
Epicuticulr wax	0.10	**0.39**	0.33
Lear rolling score	0.07	**0.66**	0.11
Digital ground cover	**0.61**	0.01	0.03
Transpiration rate	**0.65**	0.01	0.04
Net photosynthetic rate	**0.53**	0.19	0.01
Stay-green score	**0.47**	0.22	0.10
Root length	0.21	0.04	**0.56**
Grain yield/plant	**0.78**	0.04	0.01
Eigenvalue	4.75	1.58	1.22
Percent of variance	47.45	15.83	12.18
Cumulative percentage	47.45	63.29	75.46

Bold values are eigenvalues >0.50

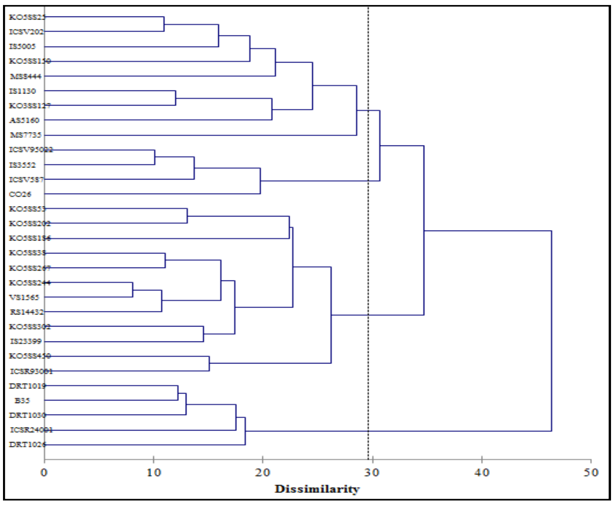

Fig 1. Pheneticdendrogram based on ten putative drought tolerance traits for thirty sorghum genotypes under drought condition

The promising genotypes with drought tolerance were identified with biplot technique from PCA analysis. The PCA for 30 individuals by three top components justified 75.46% of the total variation in the quantified putative traits (Table 7). PC1 was strongly influenced by CHY, RWC, DGC, E, P_N, SGR and GYP termed as stable yield capacity, PC2 and PC3 was mainly described by ECW, LR and RL, respectively was termed by adaptive morphological features. Accordingly, the superior tolerant genotypes could be selected based on high PC1 and PC2 values. Therefore, the current study identified four promising genotypes ICSR24001, DRT1026, DRT1030 and DRT1019 for drought tolerance (Fig 2). Similarly based on biplot from PCA analysis 12 best wheat genotypes and species were identified under drought (Pour *et al.,* 2017). Thus these genotypes could be used in sorghum breeding programmes to improve and develop new high yielding varieties with drought resistance.

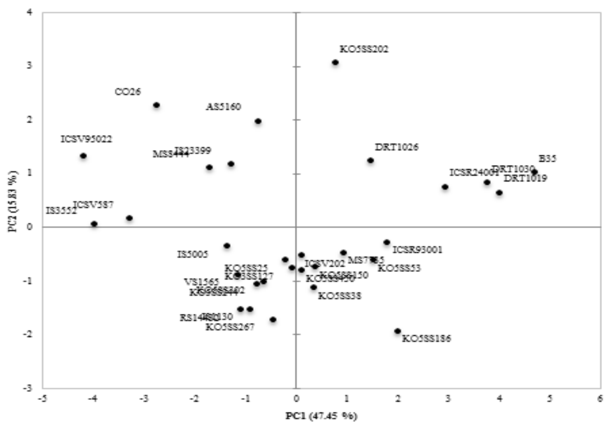

Fig 2. Biplot of thirty sorghum genotypes under drought condition

Conclusion

The pattern of genetic diversity revealed in the present study through putative physiological traits, may offer identification of novel genetic resources for improvement of drought tolerance with detailed insights for genetic resource conservation and utilization of sorghum. Although it can provide a deep understanding of drought tolerance mechanisms in sorghum subjected to further research on functional genomics in association with physiological responses.

Acknowledgement

The authors are grateful to the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore for their assistance to undertake this investigation.

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