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EXPLORING THE DIVERSITY OF CYANOGENIC POTENTIAL IN SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) AT DIFFERENT GROWTH STAGES THROUGH TREND ANALYSIS

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SUMMARY

The cultivated species of sorghum is a very high valued fodder crop. However, it cannot be used as feed until it reaches its maturity stage as its high cyanogenic potential (HCNp) poses various threats to the ruminants when fed as fodder. This research focuses on understanding the dilution pattern of dhurrin and identifying safe forage sorghum lines to avert its poisoning behaviour. Sorghum accessions were screened for HCNp through Feigl-Anger densitometry tests. Picrate paper test was performed to select ideal genotypes with low HCNp through trend analysis at various crop growth stages of 8th (seedling), 35th (vegetative) and 50th (flowering) days after sowing. The reference set accessions were evaluated for their fodder yielding traits viz., plant height, stem girth, number of tillers, number of leaves, leaf length, leaf breadth, leaf-stem ratio, green fodder yield per plant and dry fodder yield per plant. Variability and association studies showed selection for fodder potential could be focused on the traits green fodder yield, dry fodder yield per plant and plant height as they exhibited high heritability; genetic advance and strong association. Trend analysis indicated a downward trend of HCNp proving that dhurrin dilutes as the crop ages. There were genotypes performing superior to K 11, but K 11 recorded low HCN content on the 8th day; showed a gradual dilution towards 50th day and had high GFY. Consequently, K 11 was selected as the ideal genotype for low HCNp and high fodder yield. Moreover, the accessions K 3 and TKSV 1050 showed superior performance for fodder potential over the local check and could be adopted for general cultivation. Of the various methods followed the rapid screening assay proved successful for screening large number of accessions efficiently and rapidly. The traditional biochemical method though efficient is time consuming and laborious, whereas the picrate paper test is effective in screening the accessions even at field level.

Keywords: Sorghum, Fodder, Cyanogenic potential, Feigl-Anger densitometry, Picrate test

In general, sorghum is high in cyanide content and is unsafe for pasturing except after plants reach maturity and no new growth is present. Though there is natural genetic diversity for the concentration of dhurrin within sorghum lines, there have been no naturally occurring dhurrin-free genotypes identified to date. This has made the present investigation to concentrate on cyanogenic potential (HCNp) in sorghum to identify low cyanogenic lines from among 141 local germplasm accessions of Tamil Nadu.

Cases of HCN (Hydrogen cyanide) poisoning in animals feeding on sorghum forage have been reported in many parts of the country. The safe threshold limit of HCN in sorghum fodder is 500 ppm on fresh weight basis (McBee and Miller, 1980). The cyanogenic potential of sorghum is known to cause lethality to livestock when its normal growth is constrained by drought or imbalanced soil nutrition. When livestock consume forages, nitrate is normally converted in the rumen from nitrate to nitrite to ammonia to amino acid to protein. Under normal growing conditions, the intact glucoside occurs in the plant. When such plants are eaten by animals, they are readily eliminated before enough concentration occurs to be harmful. However, unusual high concentrations of nitrate obstruct the conversion and

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thereby accumulating nitrite. Nitrite is absorbed into the bloodstream directly through the rumen wall and converts haemoglobin in the blood to methemoglobin, which cannot carry oxygen. The blood turns to chocolate brown colour. An animal dying from nitrate (nitrite) poisoning actually dies from asphyxiation, or lack of oxygen. It is highly desirable that the toxicity be reduced to increase food and feed safety.

Hydrocyanic acid content is heritable and subjected to modification through selection and breeding, as well as by climate, stage of maturity, stunting of plant and type of soil and fertilizer. Good management strategies in combination with genetic variability could produce safe sorghum forage free of HCN toxin. Knowledge of various aspects of these anti nutritional factors and their effect is necessary for optimal management and utilization of forage as well as animal health.

A large segment of the rural population comprising approximately 40 million people have 2-3 cattle and 5-6 sheep per family providing 30-40% of income for livelihood. There is a need to emphasize for increased fodder production so that we could provide more support to livestock industry in the country. Demand of green fodder for rapidly expanding livestock industry is increasing day by day. Sorghum as an important fodder crop has great potential to produce high green fodder yield. The projected demand for fodder in India in 2020 is expected to be 855 MT of green fodder, 526 MT of dry fodder and 56 MT of concentrate feed (Dikshit and Birthal, 2010), as against the present demand of 666 MT of green fodder and 138 MT of dry crop residues.

Therefore, eliminating the toxicity issues through the development of sorghum varieties with highly reduced or no capacity to produce dhurrin combined with high fodder potential is a key challenge. In this outlook, the present research was initiated as a start-up by exploring the available diversity for HCNp present in sorghum accessions and discover elite genotypes possessing low HCN coupled with high fodder potential. Moreover, the investigation also involves the use of a novel technique called Feigl-Anger densitometry (FAD) for assessing the cyanogenic potential.

MATERIALS AND METHODS

The material used for the study comprised of 141 sorghum germplasm accessions collected from Agricultural Research Station (TNAU), Kovilpatti along with the local check CO (FS) 29. The investigation was conducted during 2014-15 in the Department of Plant Genetic Resources and Department of Forage crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. The methods followed; observations recorded and statistical procedures are given below.

Screening for HCNp

Seeds of 141 sorghum accessions were sown (3 seeds/well) in 96-well protrays. Composted coir pith was used as the substrate and each entry was sown in two replications. The seedlings were maintained under partial shade.

Eight-day old seedlings were screened for HCNp through a high throughput, semi-quantitative protocol, Feigl-Anger spot test modified by Takos *et al*. (2010) (Fig. 1). This test was further modified by introducing a densitometry step that involves digitally analysing the Feigl-Anger papers using MY Image Analysis v2.0 densitometry software (Rama Harinath *et al*., 2016). Yet another modification was introduced to reduce the thawing time from half an hour to 5 min while maintaining the temperature of 45^{o}C for 5 min. The accessions were categorized into low, intermediate and high HCN groups based on the mean FAD values and hence was named as Feigl-Anger Densitometry (FAD) analysis.

Trend Analysis

Twenty-four reference set accessions were raised in randomized block design with two replications along with the local check CO (FS) 29 for evaluating HCNp and fodder potential. Each accession was raised in two rows of 4 m length per replication with a spacing of 45 cm between rows and 15 cm between plants. All the recommended agronomic practices were followed during the entire crop period.

Reference set accessions were subjected for trend analysis to observe the dilution pattern of the cyanogen content, dhurrin. Trend analysis was performed at different crop growth stages of 8th (seedling stage), 35th (vegetative stage) and 50th (flowering stage) days after sowing (DAS) and the experiment was carried out in two replicates. The HCN content in 8 days old sorghum seedling was assessed using the spectrophotometric method described by Halkier and Moller (1989) and modified by Takos *et al*. (2010) in *Lotus japonicas*. The spectrophotometric method was slightly modified by subjecting the samples to a single freeze-thaw cycle against three freeze-thaw cycles recommended by Takos *et al*. (2010) to suit the sorghum crop.

The HCN content on 35 and 50 DAS (days after sowing) was analzsed by on-field evaluation of plant samples using the picrate paper method (Egan *et al*., 1998). The colour developed on the picrate papers was eluted by immersing the strips in 5 ml distilled water for 30 min and the absorbance was measured at 625 nm. By means of a standard curve prepared using KCN, a semi-quantitative measure of the amount of cyanogen present was estimated.

Statistical Analysis

The accessions were evaluated for their fodder yield and yield attributing traits such as plant height (PLH), stem girth (STG), number of tillers (NOT), number of leaves (NOL), leaf length (LEL), leaf breadth (LEB), leaf-stem ratio (LSR), green fodder yield per plant (GFY) and dry fodder yield per plant (DFY) The traits were recorded in five randomly chosen plants to evaluate the fodder potential.

Quantitative data of fodder yield traits was statistically analyzed for genotypic variance, phenotypic variance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance. Genotypic and phenotypic correlation coefficients were computed from the mean of fodder yield traits besides HCNp for all combinations of characters by using the statistical package Genetical Researchers (GENRES) v.7.01 software.

RESULTS

High Throughput Screening for HCNp

FAD mean values for the 141 sorghum accessions ranging from 3.5 (TKSV 1126) to 39.3 (IS 9807) were obtained from the densitometry analysis (Appendix 1). The accessions were categorized as low (0-10 mean FAD value), intermediate (10-30 mean FAD value) and high (30-40 mean FAD value) HCN groups. Low group consisted of 12 accessions, intermediate group had 93 accessions and high HCNp group comprised 36 accessions. Representative accessions from each group of low to high were chosen in such a way that it exhibits normal distribution and thus 24 accessions (Table 1) were shortlisted. The reference set constituted six accessions from low HCNp group; 12 accessions from intermediate group and six accessions from high HCNp group.

Trend Analysis

The local check CO (FS) 29 possessed low HCN content of 632 ppm succeeded by TKFS 1049 (653 ppm) and K 11 (728 ppm). The accession TKSV 1130 recorded the highest concentration of 1838 ppm followed by K 3 (1830 ppm) and TKSV 1171 (1809 ppm) and were statistically on par (Table 2). Significantly inferior genotypes for HCN were selected to identify low HCN lines. A total of 13 accessions were identified to possess significantly low HCN content at 8 DAS, while 20 and 18 accessions showed significant low levels of HCN at 35 and 50 DAS respectively. The accession TNS 623 showed low HCN concentration of 18 ppm at 35 DAS while K 11 recorded the highest concentration of 218 ppm at the 35th day.

At the age of 50 DAS, by which most of the entries started flowering, the level of HCN in almost all the entries was evidently low and four accessions *viz*., TKFS 11107, TNS 623, TKFS 1051 and TKSV 1046 had very low concentration of 6 ppm. The data indicated that HCN content of all 24 sorghum accessions at maturity were found to be under safe limit of 500 ppm.

Statistical Analysis

Analysis of variance for the fodder yield traits showed significantly high differences among the genotypes for all the characters. This indicated the presence of considerable variability among the experimental material studied.

Plant height, GFY and DFY were found to be promising in the accession K 3 with 346.10 cm, 1452.40 g and 262.30 g respectively (Table 3). The accession TKSV 1050 had high NOT with a mean of 4.20. TKFS 1198 showed high mean for NOL (13.60) and LEL (92.90 cm) while LEB was high in IS 18551 with a mean of 10.24 cm.

The genetic parameters *viz*., GCV and PCV, heritability and genetic advance as per cent mean for fodder yield traits are graphically represented (Appendix 2) for the nine fodder yield and yield attributing traits studied. The trait, NOT exhibited high PCV, GCV, heritability and genetic advance as per cent mean of 60, 59.97, 99.91 and 123.49 per cent respectively. The traits GFY and DFY also expressed high estimates of the genetic parameters under study.

Correlation analysis was assessed among the nine fodder traits besides HCNp with GFY as the dependent variable (Table 4). Genotypic correlation showed that GFY had positive and high significant association with PLH (0.851), NOT (0.813) and DFY (0.944). The traits NOL (0.424) and LEL (0.350) showed positive and significant association. Phenotypic correlation exhibited positive and high significant association for GFY with PLH (0.783), NOT (0.785) and DFY (0.885). The trait LEB showed positive and high significant association with HCNp (0.474).

APPENDIX 1
List of accessions across different HCNp groups based on mean FAD

Category	Accession No.	FAD mean	Accession No.	FAD mean	Accession No.	FAD mean
Low	TKSV 1126	3.5	TKSV 1161	6.6	TKSV 1133	9.6
	TKSV 1166	5	K 11	7.8	TKSV 1147	9.9
	TKSV 1168	5.1	TKSV 1116	8.1	TKSV 1181	9.9
	TKFS 1050	6.4	TKFS 11107	8.7	A 522	10
Intermediate	TKSV 1182	10.3	TKSV 1179	19.8	TKSV 1129	23.8
	TKFS 1194	10.5	TKFS 11101	19.9	TKSV 1163	23.8
	TKFS 1051	12.1	TKSV 1047	20.2	TKSV 1045	23.9
	TKSV 1120	13.1	TKSV 1028	20.8	CSV 23	24.2
	TKSV 1156	13.1	TKSV 1158	21.1	TKFS 11106	24.3
	TKSV 1008	13.2	TKSV 1171	21.1	CSV 20	24.3
	TKSV 1132	13.9	TKSV 1141	21.2	TKSV 1115	24.4
	TKSV 1127	14	TKSV 1041	21.3	TKSV 1121	24.4
	TKFS 11100	14.6	TKSV 1180	21.3	SPV 2117	24.9
	TNS 624	14.6	TKSV 0906	21.3	R 821	24.9
	TKSV 1105	15	TKFS 11112	21.5	CSV 17	25.1
	TKSV 1164	15	TKSV 1122	21.6	TKSV 1112	25.3
	TKSV 1102	15.4	TKSV 1131	21.6	SPV 2116	25.6
	TKSV 1174	15.4	TKFS 1049	21.8	TKSV 1150	26.1
	TNS 623	15.5	TKFS 11102	21.8	TNS 638	26.2
	TKSV 1172	15.7	TKSV 0902	22.1	TKSV 1101	26.2
	IS 2663	16.4	K 3	22.2	SPV 2115	26.6
	TKSV 1043	16.4	TKSV 1001	22.4	TKSV 1170	26.7
	TKSV 1109	17.3	C 43 - 81	22.6	TKFS 1195	26.9
	TKSV 1123	17.3	TKSV 1004	22.6	TKSV 1040	26.9
	Chitra	17.5	TKSV 1005	22.7	TKSV 1023	27.2
	TKSV 1159	18	TKSV 1034	22.7	Uthra	27.4
	TKSV 1014	18.1	IS 3201	23	TKSV 1130	27.4
	Anuradha	18.4	TKSV 1107	23	TKSV 1145	27.4
	TKSV 1033	18.9	TKFS 11104	23.1	TKFS 1197	27.6
	TKSV 1162	19.3	TKSV 1149	23.3	SPV 2110	28.4
	TKSV 1175	19.3	TKSV 1103	23.4	TKSV 1022	28.5
	TKSV 1106	19.4	TKFS 204	23.5	TKFS 1196	28.8
	TKSV 1046	19.7	TKSV 1165	23.6	TKFS 11108	29.5
	TKSV 1157	19.7	TKSV 1173	23.6	TKSV 1021	29.8
	CSV 15	19.8	TKSV 0809	23.6	SPV 2121	29.9
High	TKFS 11111	30.1	SPV 2112	31.5	TKSV 1039	33.5
	TKSV 1117	30.1	TKSV 1031	31.5	SPV 2125	33.8
	TKFS 11110	30.3	TKSV 1003	31.6	IS 18758	33.8
	TKSV 1010	30.3	TKSV 1037	32.1	IS 2660	34.1
	TKFS 1198	30.5	SPV 2123	32.3	TKSV 1036	34.1
	TKFS 11109	30.5	TKSV 1144	32.3	SPV 2113	34.4
	TKFS 11103	30.6	TKFS 1052	32.4	IS 7034	34.6
	B 35	30.7	SPV 2122	32.4	Kallipatti	35.1
	TKSV 1016	31	SPV 2114	32.9	TKSV 1030	35.5
	TKSV 1029	31.1	TKSV 1026	33.2	IS 18551	36
	K 5 - 80	31.2	TKSV 113	33.2	TAM 428	36.9
	TKSV 1135	31.4	TKSV 1038	33.4	IS 9807	39.3

DISCUSSION

FAD Screening

Quantitative assessment of HCNp through the conventional biochemical tests is laborious and time consuming. Therefore, a simple rapid screening test, Feigl-Anger spot test was used for screening large samples of sorghum accessions with considerable modifications. This method is faster, and capable enough to analyze 96 samples in a microtiter plate at a time. In the present study, Feigl-Anger spot test was combined with a computational image analysis process and together was referred as Feigl-Anger densitometry analysis (FAD).

The FAD method is the first of its kind followed for sorghum. The densitometry analysis made the semi-quantitative test more reliable and

TABLE 1
Shortlisted accessions based on mean FAD values

Category	Accession No.	Mean FAD value
Low (0-10)	TKSV 1126	3.5
	TKSV 1166	5.0
	TKSV 1050	6.4
	TKFS 1161	6.6
	K 11	7.8
	TKFS 11107	8.7
Intermediate (10-30)	TKSV 1133	10.1
	TKSV 1182	10.3
	TKSV 1127	14.0
	TNS 623	15.5
	TKFS 1051	12.1
	TKSV 1123	17.3
	TKSV 1046	19.7
	TKSV 1171	21.1
	TKFS 1049	21.8
	K 3	22.2
	TKSV 1115	24.4
	TKSV 1130	27.4
High (30-40)	TKFS 11111	30.1
	TKFS 1198	30.5
	SPV 2123	32.3
	IS 18758	34.1
	IS 18551	36.0
	TAM 428	36.9

accountable than a mere visual classification. This is supported by our previous work where Rama Harinath *et al*. (2016) used FAD analysis for the first time to screen 232 sorghum germplasm accessions. The present investigation also proved that the FAD analysis was effective in screening the sorghum accessions. The additional modification of introducing hot air oven to reduce the thawing period from half an hour to 5 min provided reliable and substantial results.

Trend Analysis

HCN is an anti-nutritional factor in forage sorghum which is potentially toxic to animals when fed with 30-35 days old crop. To select ideal genotypes with low HCNp, trend analysis was performed. Similar kind of selection was done by Chaturvedi *et al*. (1994) who grouped 20 genotypes as "unsafe" for feeding to cattle at the flowering stage. HCN content was determined at 8, 35 and 50 DAS.

The cyanide content of sorghum increases rapidly during early growth stage, after which it declines with increase in plant age (Pandey *et al*., 2011). Therefore, young seedlings (8 days old) were subjected for spectrophotometric estimation to reveal the maximum HCNp of the accessions (Table 2). Shoot

TABLE 2
Mean values of HCN content (ppm) estimated during various crop growth stages

S. No.	Accession No.	8th day (ppm)	35th day (ppm)	50th day (ppm)
1.	TKSV 1126	1379	116	**56**
2.	TKSV 1166	1329	64	28
3.	TKSV 1050	994	36	30
4.	TKFS 1161	1551	58	46
5.	K 11	728	**218**	22
6.	TKFS 11107	1060	54	6
7.	TKSV 1133	928	174	30
8.	TKSV 1182	966	56	32
9.	TKSV 1127	1445	70	12
10.	TNS 623	1780	18	6
11.	TKFS 1051	1601	58	6
12.	TKSV 1123	1531	64	32
13.	TKSV 1046	1420	90	6
14.	TKSV 1171	1809	138	36
15.	TKFS 1049	**653**	38	12
16.	K 3	1830	74	10
17.	TKSV 1115	1301	38	26
18.	TKSV 1130	**1838**	70	40
19.	TKFS 11111	1163	98	50
20.	TKFS 1198	1248	38	16
21.	SPV 2123	1188	80	20
22.	IS 18758	1488	46	24
23.	IS 18551	1627	76	38
24.	TAM 428	1621	80	28
Check	CO (FS) 29	632	74	20
Grand mean		**1324.34**	**77.04**	**25.28**
S. Ed		**53.30**	**12.65**	**4.02**
C. D. (P=0.05)		**105.79**	**25.17**	**8.00**

Lowest mean values are indicated in bold and underlined and highest mean values are indicated in bold.

tips were used for conducting the experiment as reported by Halkier and Moller (1989), who confirmed the presence of cyanogenic glycoside content in the tip of young seedlings of *Sorghum bicolor*. At the age of 8 days the accessions TKSV 1130, K 3 and TKSV 1171 were considered hazardous as they expressed high HCNp. Abusuwar and Hala (2010), Pandey *et al*., (2011) and Sarfraz *et al*., (2012) reported higher HCN content in Abu Sabein (hybrid variety), Hegari and local sorghum. These accessions were found to be lethal for grazing and may likely to poison livestock during their young stages of growth. On the flip side two accessions *viz*., TKFS 1049 and K 11 reported very low cyanogenic contents of 653 and 728 ppm respectively but exceeded the safe limit of 500 ppm and therefore they may not be used as a sole source of fodder during young growth stages. Our findings were well in accordance with earlier studies reported by Wu

and Wei (1989). They measured HCNp in 148 sorghum and Sudan-grass (*Sorghum sudanense*) varieties during seedling growth. The varieties with the lowest HCNp were Xinliang 80 (672 ppm), Sudancao (753 ppm), Huangke Sudancao (856 ppm), Limuji (860 ppm) and MI03 (876 ppm).

Assessment of HCN content at 35 and 50 DAS were done directly in the field to minimize error in assessment of HCN content. Picrate strips were used for this purpose (Egan *et al*., 1998). Pandey *et al*. (2011) attempted similar type of work on picrate paper expressing intensity of colour development by the leaf tissues of sorghum accessions. Though the accessions TKFS 11107, TNS 623, TKFS 1051 and TKSV 1046 started with varied HCN content at 8th day, they ended with very low HCN levels at 50th day and were identified as near acyanogenic lines. These genotypes were considered as highly safe for the cattle to feed. Earlier findings by Kumar *et al*. (2011) and Pandey *et al*. (2011) showed the occurrence of near acyanogenic lines. Their experiments on sorghum varieties Dichuniyo, Vadgam, Dhanera, Malavan recorded 15.3, 5.01, 6.57, 8.79 mg % on DM basis at 50 DAS.

The entire set of accessions contained HCN contents lesser than the critical limit at 50 DAS and were considered safe for livestock feeding. Similar observations were reported by Sarfraz *et al*. (2012). Unexpectedly all the accessions studied were under the safe limits of HCN at 35 DAS which signifies the use of these lines for feed purposes as need arises earlier than the accustomed 50th day when the crop is grown under favourable conditions and not subjected to any abiotic stress.

Mean Performance

Plant height is an important growth parameter which influences fodder quantity, quality and mostly

TABLE 3
Mean performance of accessions for fodder yield and yield related traits

S. No.	Accessions	PLH (cm)	STG (cm)	NOT	NOL	LEB (cm)	LEL (cm)	LSR	GFY (g)	DFY (g)
1.	TKSV 1126	200.80	1.52	1.00	9.40	8.80	76.30	0.23	566.60	79.19
2.	TKSV 1166	176.80	1.60	1.00	9.80	9.18	77.90	0.24	516.70	76.22
3.	TKSV 1050	288.90	1.72	**4.20**	10.40	9.86	89.00	0.22	1409.47	213.35
4.	TKFS 1161	168.10	1.70	1.00	9.60	9.02	75.10	0.19	**358.80**	51.69
5.	K 11	324.10	**1.50**	2.60	12.80	**7.90**	87.10	0.20	1131.50	210.77
6.	TKFS 11107	296.70	1.52	1.60	13.00	9.38	84.40	0.20	887.10	122.45
7.	TKSV 1133	170.00	1.88	1.00	10.00	8.92	81.10	0.26	583.37	110.77
8.	TKSV 1182	175.60	1.92	1.00	9.80	8.92	66.00	0.24	691.53	68.74
9.	TKSV 1127	229.60	1.68	1.00	12.20	9.06	71.90	0.24	744.40	112.45
10.	TNS 623	209.00	1.64	1.00	10.40	9.44	86.20	0.24	575.27	100.69
11.	TKFS 1051	287.90	1.70	3.40	13.40	8.22	**70.20**	0.17	1117.07	204.92
12.	TKSV 1123	197.60	2.26	1.00	11.60	9.36	82.30	0.22	858.80	128.35
13.	TKSV 1046	**136.80**	1.86	1.00	**9.20**	8.50	75.40	0.19	437.67	63.67
14.	TKSV 1171	217.70	**2.28**	1.00	9.80	9.54	70.50	0.20	443.63	**51.19**
15.	TKFS 1049	321.90	1.70	2.60	10.80	8.04	88.10	0.15	1223.80	178.35
16.	K 3	**346.10**	1.72	3.60	11.20	9.02	80.10	0.20	**1452.40**	**262.30**
17.	TKSV 1115	198.40	2.24	1.00	12.20	8.92	78.50	0.21	777.40	111.65
18.	TKSV 1130	197.10	1.96	1.00	12.60	9.36	77.30	0.24	725.23	89.22
19.	TKFS 11111	279.40	2.18	2.00	9.80	9.52	82.70	0.12	1137.90	222.58
20.	TKFS 1198	288.90	1.82	1.80	**13.60**	8.04	**92.90**	0.26	850.90	167.55
21.	SPV 2123	239.20	1.62	1.00	12.80	9.62	73.40	0.17	1194.33	176.55
22.	IS 18758	187.00	2.00	1.00	12.40	9.32	80.80	**0.28**	699.47	105.13
23.	IS 18551	255.80	1.96	1.00	10.80	**10.24**	88.20	0.20	733.60	87.76
24.	TAM 428	210.00	1.94	2.20	12.20	9.40	85.10	0.23	768.10	160.75
Grand mean		**233.48**	**1.83**	**1.63**	**11.24**	**9.07**	**80.02**	**0.21**	**828.54**	**131.51**
C. D. (P=0.05)		**21.08**	**0.23**	**0.04**	**1.08**	**0.88**	**5.93**	**0.03**	**101.44**	**15.56**
CV %		**7.19**	**9.91**	**1.76**	**7.67**	**7.77**	**5.90**	**11.57**	**9.75**	**9.42**

Highest mean values are indicated in bold and underlined, and lowest mean values are indicated in bold.
PLH - Plant height, STG - Stem girth, NOT - Number of tillers/plant, NOL - Number of leaves/plant, LEL - Leaf length, LEB - Leaf breadth, LSR - Leaf-stem ratio, GFY - Green fodder yield/plant and DFY - Dry fodder yield/plant.

TABLE 4
Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficient

Traits	PLH	STG	NOT	NOL	LEB	LEL	LSR	DFY	HCNp	GFY
PLH	**1.000**	-0.321	0.772**	0.457*	-0.243	0.494**	-0.469*	0.831**	-0.272	0.851**
STG	-0.254	**1.000**	-0.279	-0.130	0.330	-0.117	-0.000	-0.189	0.313	-0.189
NOT	0.742**	-0.215	**1.000**	0.238	-0.276	0.364*	0.341*	0.839**	-0.208	0.813**
NOL	0.364*	-0.055	0.199	**1.000**	-0.268	0.239	0.132	0.436*	0.007	0.424*
LEB	-0.158	0.291	-0.16	-0.118	**1.000**	-0.035	0.072	-0.228	0.474**	-0.091
LEL	0.387*	-0.061	0.297	0.137	0.051	**1.000**	0.037	0.422*	-0.269	0.350*
LSR	-0.390*	-0.045	-0.281	0.065	-0.003	0.036	**1.000**	-0.433*	0.192	-0.464*
DFY	0.782**	-0.153	0.822**	0.354*	-0.156	0.341	-0.344*	**1.000**	-0.263	0.944**
HCNp	-0.25	0.241	-0.202	-0.001	0.277	-0.206	0.14	-0.255	**1.000**	-0.338
GFY	0.783**	-0.163	0.785**	0.336	-0.081	0.278	-0.373*	0.885**	-0.309	**1.000**

*,**Significant at P=0.05 and P=0.01 levels, respectively.
PLH - Plant height, STG - Stem girth, NOT - Number of tillers/plant, NOL - Number of leaves/plant, LEL - Leaf length, LEB - Leaf breadth, LSR - Leaf-stem ratio, GFY - Green fodder yield/plant and DFY - Dry fodder yield/plant.

shows relative vigour of the crop. Long slender fine stems are often preferred by animals than short thick stems as they affect palatability of the forage. K 3 obtained the highest mean for PLH, but it did not significantly differ with K 11 and TKFS 1049. With respect to height, generally tall plants yielded more than short types. The earlier studies conducted by Nabi *et al*. (2006) and Ayub *et al*. (2010) for sorghum cultivars also supported our findings for PLH.

Green leaves contribute much to the forage quality. NOL and LEL were high in the genotype TKSV 1198. However, the values recorded for NOL in genotypes TKSV 1198, TKFS 1051 and TKFS 11107 were statistically at par. It is obvious that tall plants produce more number of leaves and *vice-versa*. This was evident from our findings (Table 3). K 11 which had a plant height of 324.10 cm registered a high leaf count of 12.80, while the genotype TKSV 1046 which recorded low plant height (136.80) reported less number of leaves with a mean of 9.20. The significant differences among sorghum cultivars reported by Nabi *et al*. (2006) supported our findings.

High nutritive value for most forages is found in the leaf, which is considered a desirable characteristic. In general, the most nutritious grasses are those which have a high proportion of leaf to stem and maintain this high proportion even when nearly mature. The genotype IS 18758 was found to have the maximum proportion of leaf to stem and was statistically on par with TKFS 1198 and TKSV 1133. The genotype K 3 recorded highest GFY of 1452.4 g. However, it could not produce significantly higher GFY over the genotype TKSV 1050 (1409.47 g). Similar findings were reported by Ghasemi *et al*. (2012) and Sarfraz *et al*. (2012). It is evident that if there is higher GFY, there would be higher DFY. Therefore, DFY too was recorded the highest in K 3. Similar observations were reported by Carmi *et al*. (2006) and Nabi *et al*. (2006).

As discussed above, earlier researchers stated that tall plants had slender stems; high number of leaves and yielded more than short types. In conclusion, the genotype K 3 justified these statements with high plant height, slender stems, more leaves and

APPENDIX 2
Genetic analysis for fodder yield traits in the reference set

S. No.	Characters	PV	GV	PCV	GCV	Heritability	GA as % mean
1.	PLH	3579.71	3298.13	25.63	24.60	92.13	48.64
2.	STG	0.08	0.05	15.71	12.19	60.20	19.48
3.	NOT	0.96	0.96	60.00	59.97	99.91	123.49
4.	NOL	2.60	1.86	14.35	12.13	71.44	21.11
5.	LEB	0.74	0.25	9.51	5.49	33.34	6.53
6.	LEL	65.39	43.07	10.11	8.20	65.86	13.71
7.	LSR	0.002	0.001	20.11	16.45	66.88	27.71
8.	GFY	99670.73	93151.17	38.10	36.84	93.46	73.36
9.	DMY	3772.12	3618.63	46.70	45.74	95.93	92.29

high biomass. Henceforth, the genotype K 3 which is also a released variety could be utilized in breeding programmes for these desired qualities.

Genetic Variability Studies

The estimates of PCV were greater than GCV for all the traits denoting environmental factors influencing their expression to some degree or other (Jain *et al*., 2011; Jain and Patel, 2013; Ghorade *et al*., 2015). The narrow differences between PCV and GCV suggested their relative resistance to environmental alterations. Among the traits, GCV and PCV were high for NOT, DFY and GFY. The high values of GCV and PCV for these traits suggested that there is a possibility of improvement through direct selection for the traits (Kumar, 2014). It was medium to low for other traits *viz.*, STG, NOL, LEL and LEB. Similar findings were reported in the studies conducted by Jain and Patel (2013).

High heritability accompanied with high expected genetic advance for the characters suggest that the genes governing these characters may have additive effect (Jain *et al*., 2009). It can be mentioned here that the traits NOT, followed by DFY and GFY exhibited high heritability coupled with high genetic advance and high GCV indicating that these characters are controlled by additive gene action and phenotypic selection for these characters would be effective. Our findings were in parallel with that of Jain and Patel (2013), Kumar (2014) and Ghorade *et al*. (2015).

Association of Traits

Knowledge of association between yield and its component traits and among the component parameters themselves can improve the efficiency of selection in plant breeding. When there is positive association of major yield characters and its components breeding would be very effective but when these characters are negatively associated, it would be difficult to exercise simultaneous selection for them in developing a variety (Nemati *et al*., 2009).

The genotypic correlation coefficients were higher than phenotypic correlation coefficients. Similar observations had earlier been reported by Alhassan *et al*. (2008). Expression of higher genotypic correlation than phenotypic correlation is indication of strong inherent relationship between these characters. PLH, NOT, NOL, LEL and LSR were positively and significantly associated with GFY and DFY. It is obvious that when the number of leaf is many, there will be a greater surface area for photosynthesis; greater photosynthesis can translate into more photosynthates, ultimately resulting in increased fodder yield (Alhassan *et al*. 2008). It is also noticed that the characters that exhibited positive associations with fodder yield have also showed positive associations among themselves (Table 4). HCNp showed a strong positive and significant association with LEB. It is a known fact that cyanogenic glucoside dhurrin is present in the leaf tissues. Muthuswamy *et al*. (1976) reported that HCN was more in shoot tissue (leaves and stem) and this indicates that when the leaf area is high there will be more cyanoglucoside content.

Based on the mean performance of the accessions with respect to various fodder yield traits, K 3 was found to be superior in terms of PLH, GFY, DFY, NOT and NOL which could be very well used as a donor in hybridization programme to develop high biomass yielding sorghum genotypes. Similarly, another released variety K 11 was also superior in most of the fodder yield traits in addition to low HCNp, proving its worthiness as a parent in developing low cyanogenic lines coupled with high fodder yield. On the basis of genetic variability and association studies the traits GFY, DFY and PLH were having high broad sense heritability with high expected genetic advance, strong association and therefore, could be focused in selection programme. Similar results have been reported by earlier workers (Jain *et al*. 2009; Jain *et al*. 2011; Kumar, 2014).

Methods for Cyanogen Assessment

In the present investigation, HCNp of sorghum was estimated using three methods viz., Feigl-Anger densitometry (FAD), spectrophotometric method and picrate paper test. The FAD method was semi-quantitative, while the spectrophotometric method was a quantitative method. The picrate paper test was advantageous owing to its qualitative and quantitative nature.

The FAD method was beneficial over the other methods in screening large number of samples at a time (requires a short reaction time) but the intensity of blue colour thus developed was not stable over time and stressed the need for proper storage. This was supported by the earlier works made by Egan *et al*. (1998). The second method (spectrophotometric method) was efficient and reliable in estimating the HCN content quantitatively in the young seedlings but was found to

be ineffective while using aged plant samples.

The picrate paper test proved to be advantageous as it was versatile in testing the cyanogenic samples under field conditions as well as in controlled environment. It also had an added advantage of eluting the colour developed and determining the intensity spectrophotometrically. Yet, ineffective in handling larger samples. Our observations were supported by similar findings obtained by Egan *et al*. (1998) and Avais *et al*. (2011).

Overall, the picrate paper test proved productive in determining the cyanide content present in the sorghum crop as we noticed four advantages viz., (i) colour developed is more stable than the FAD method, (ii) colour may be eluted and it could be measured quantitatively, (iii) alkaline picrate solution is less hazardous than the tetra base (FAD) chemical which is carcinogenic and (iv) could be used under field conditions as a qualitative method. Egan *et al*. (1998) also highlighted the advantages of picrate paper test. Though this method was efficient it lagged behind the FAD method in few aspects viz., time consumption and screening large samples. In conclusion, the FAD method can be used as a screening technique while the picrate paper test could be followed as an alternate for the traditional spectrophotometric method for determining the HCN content in sorghum crop.

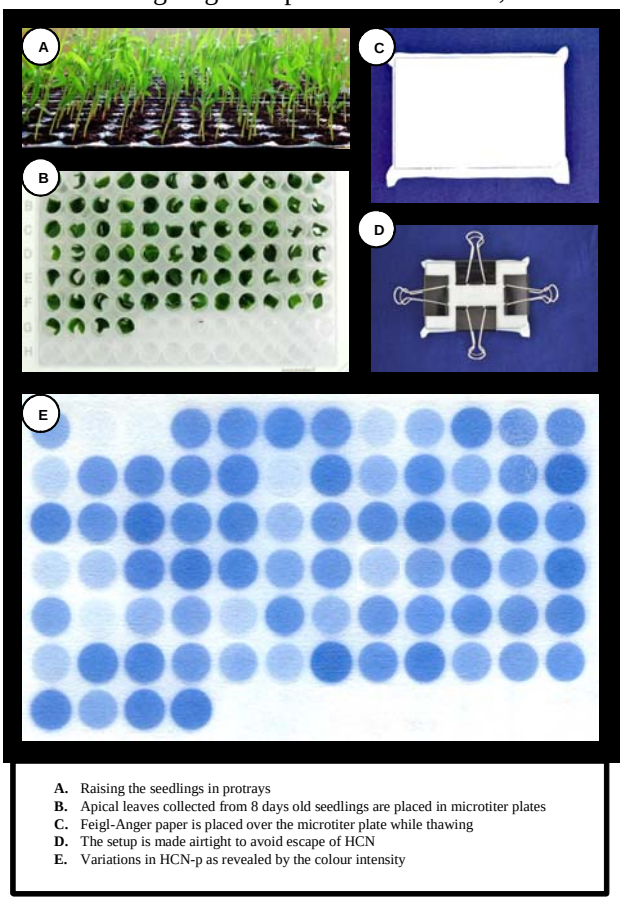

Fig. 1. Various steps involved in high throughput screening for HCNp in sorghum using Feigl-Anger paper.

CONCLUSION

Under the light of present study, the superiority of K 3 and TKSV 1050 over the other genotypes suggests their adoption for general cultivation owing to their superior performance for fodder yield over the local check CO (FS) 29. The accessions with negligible HCN content could well be exploited through crop improvement programme. There were genotypes performing superior to K 11, but K 11 recorded low HCN content on the 8th day; showed a gradual dilution towards 50th day; and had high GFY. Consequently, the variety K 11 was selected as the ideal genotype for low HCNp and high fodder potential. The accessions TKFS 11107, TNS 623, TKFS 1051 and TKSV 1046 obtained negligible cyanide content and were taken as near acyanogenic. Hybridization could be attempted between low cyanogenic lines and high fodder yielding accessions to develop genotypes of low HCNp and higher biomass. The accessions shortlisted from this study are stored in Ramiah Gene Bank, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. These accessions which have been identified as to have low HCNp should be subjected for stability analysis and location trials as HCNp tends to get affected by various environmental factors. This would throw more light on this subject.

Future Scope

- Using the cyanide detection methods followed in the present work HCN profiling of leading sorghum varieties and hybrids can be made to suggest the right time of harvest to the farming community.
- The low cyanogenic lines could be studied at molecular level for successfully developing acyanogenic cultivars.

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