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Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
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Development of a reference set of sorghum (*Sorghum* spp.) for cyanogenic potential (HCN-p) and evaluating their fodder yield traits

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Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) the fifth most important cereal crop of the world is also valued for its fodder and stover. In India, fodder sorghum is grown in 2.6 mha mainly in western UP, Haryana, Punjab, Rajasthan and Delhi and fulfils over two-third of the fodder demand.

Sorghum has four desirable qualities *viz.*, high dry matter yield, light, water and nitrogen use efficiency. Sorghum stover is valued over all other sources of fodder. Thus, sorghum is used as fodder to the domestic animals for its better performance. The projected demand for fodder in India in 2020 is expected to be 855 MT of green fodder, 526 MT of dry matter and 56 MT of concentrate feed (Dikshit and Birthal, 2010). In this perspective it is important to develop genotypes of high fodder yield and nutritive value.

Cases of cyanide poisoning in animals feeding on sorghum forage have been reported in many parts of the country. HCN is absorbed into the blood stream very quickly and inhibits the animal's ability to deliver oxygen to tissue for cellular respiration. According to the Merck Veterinary Manual, the minimum lethal blood concentration level for HCN is approximately 3.0 µg/ml or less. It is highly desirable that the toxicity of cyanogenic plants to livestock be reduced. This is achievable by selective breeding, screening of germplasm for low-HCN-p, mutagenesis and genetic engineering.

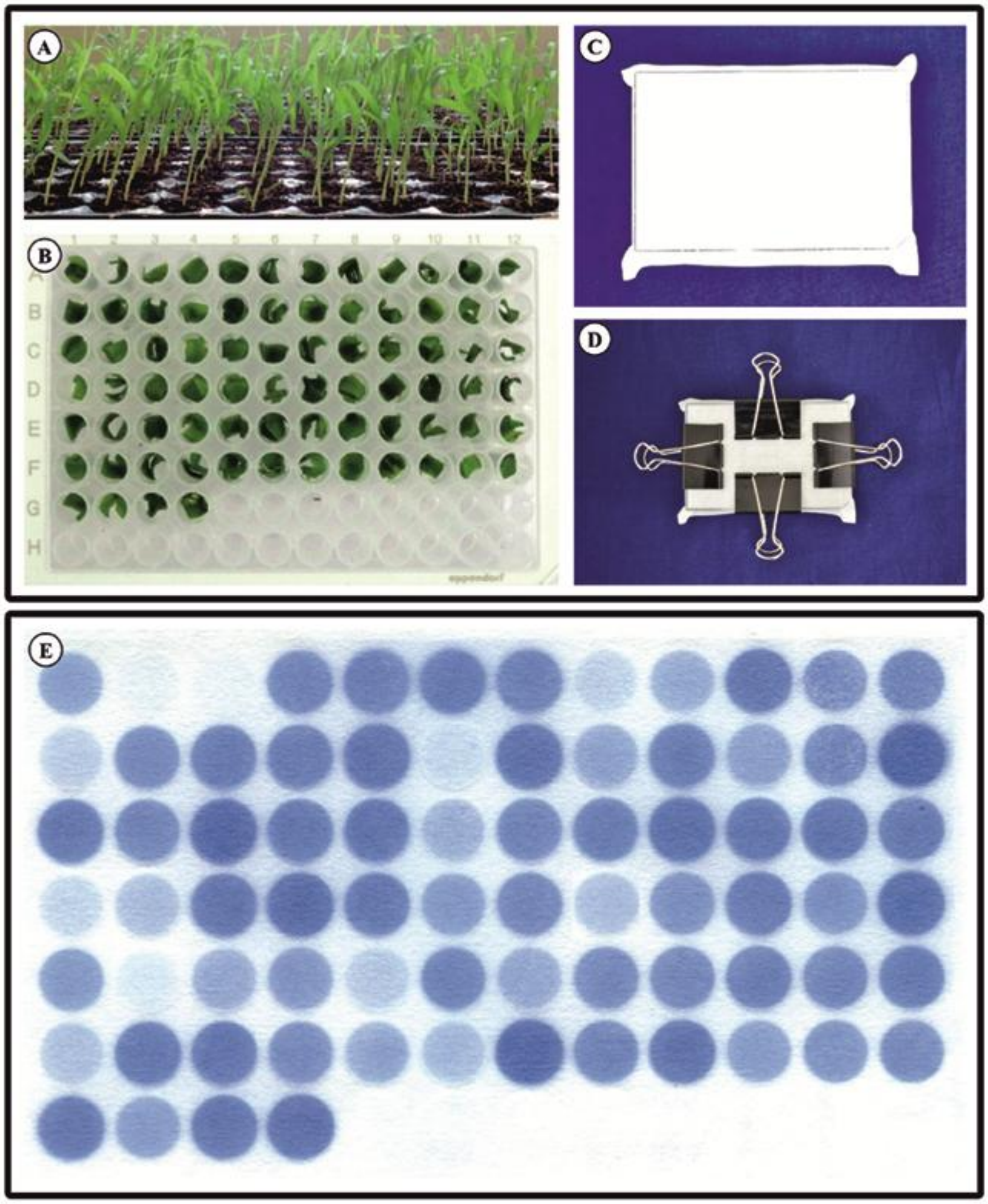
With this background, the present investigation was contemplated among sorghum germplasm accessions to develop a reference set for cyanogenic potential (HCN-p). These accessions were also evaluated for fodder yield traits and entries with low HCN-p and high biomass were identified.

Materials and Methods

A total of 141 sorghum germplasm accessions collected from Agricultural Research Station, Kovilpatti, Tamil Nadu was subjected for this study in the Department of Plant Genetic Resources, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. These materials were screened for their HCN-p through Feigl-Anger Densitometry (FAD) analysis and the shortlisted entries were subjected for studying the dilution pattern of HCN using picrate paper method. Path analysis was done using GENRES software to identify each character's contribution to the diversity of the reference set, keeping HCN-p as the dependent variable.

Feigl-Anger densitometry (FAD) analysis: All the 141 germplasm accessions were raised in portrays and the 8th day old seedlings were subjected for HCN-p screening by adopting the rapid high-throughput qualitative protocol suggested by Kakes (1991) and further modified by Takos *et al.* (2010). Dried Feigl-Anger paper was prepared by dipping Whatmann No.1 filter paper in a solution made by mixing equal volumes of 1% copper ethylacetoacetate and 1% 4, 4'-methylenebis in chloroform. Apical leaves from eight days old seedlings were placed in microtiter plates and were frozen at -20^{o}C for one hour. A feigl-anger paper was placed over the plate immediately after freezing and was rapidly thawed at 45^{o}C in hot air oven. Due to rapid thawing, freezing injury occurs in the leaf tissues. Due to the injury, cyanide and the glycosidic enzyme interact to release HCN, which will be captured by the Feigl-Anger paper. As a result of which individual blue colour spots develop over each sample within five minutes. The Feigl-Anger papers were scanned and the images were subjected for analysis using the densitometry software to group the accessions based on colour intensities into different categories of low, intermediate and high HCN-p. A total of 24 accessions selected across these categories were established as reference set and raised in the field, along with the local check CO (FS) 29 for studying trend analysis and fodder yield potential. The step-wise procedure is illustrated in fig. 1.

Figure 1. High throughput screening for HCNp in Sorghum using Feigl-Anger paper



A. Raising the seedlings in portrays
B. Apical leaves from 8th day old seedlings are placed in microtiter plates
C. Feigl-Anger paper is placed over the microtiter plate
D. The setup is made airtight to avoid escape of HCN
E. Variations in HCN-p indicated by the colour intensity

Dilution trend analysis: A trend analysis was performed by on-field evaluation of plant sample using picrate paper (Bradbury *et al*., 1998) to understand the dilution pattern of the HCN-p in the plant sample at different crop growth stages of 8^{th}, 35^{th} and 50^{th} day from sowing.

Observations recorded: The fodder yield and attributing traits such as plant height, stem girth, number of tillers, number of leaves, leaf length, leaf breadth, leaf-stem ratio, green fodder yield per plant and dry matter yield per plant besides cyanogenic potential (HCN-p) were recorded in five randomly chosen plants.

Results and Discussion

Feigl-Anger densitometry analysis: Based on the densitometry analysis, 23 lines were identified as low, 81 as intermediate and 37 as high HCN-p types. Screening a large number of samples using quantitative tests has been laborious and time consuming. Therefore, simple and rapid screening protocols were developed through picrate paper by Bradbury *et al*., (1998) and Feigl-Anger test proposed by Feigl-Anger (1966). The Bradbury's picrate paper method requires longer incubation period while the Feigl-Anger requires less than an hour to develop the colour.

Rama Harinath (2012) used this Feigl-Anger test for the first time to screen 232 sorghum germplasm accessions. The present investigation proved that the FAD method was found to be as useful in rapid screening and identifying 24 accessions as reference set from a total of 141 accessions.

Trend analysis at different crop growth stages: The results of trend analysis made at different crop growth stages are furnished in Table 1. The values of HCN-p tested at 8th day ranged from 653 ppm in TKFS 1049 to 1837 ppm in TKSV 1130.

Table.1 Trend analysis for HCNp at different crop growth stages

S.NO.	ACCESSION NO.	8TH DAY (PPM)	35TH DAY (PPM)	50TH DAY (PPM)	GFY/Plant
1.	TKSV 1126	1379	58	25	566.60
2.	TKSV 1166	1389	32	11	516.70
3.	TKSV 1050	994	18	12	**1409.47**
4.	TKFS 1161	1550	29	20	358.82
5.	K 11	728	**109**	8	1131.57
6.	TKFS 11107	1060	27	**0**	887.16
7.	TKSV 1133	927	87	15	580.03
8.	TKSV 1182	965	28	13	691.53
9.	TKSV 1127	1441	35	6	744.49
10.	TNS 623	1780	**9**	**0**	576.27
11.	TKFS 1051	1600	29	**0**	1117.07
12.	TKSV 1123	1530	32	13	858.80
13.	TKSV 1046	1420	45	**0**	437.67
14.	TKSV 1171	1809	69	18	444.63
15.	TKFS 1049	**653**	19	6	1223.80
16.	K 3	1830	37	2	**1452.41**
17.	TKSV 1115	1300	19	10	777.47
18.	TKSV 1130	**1837**	35	17	725.23
19.	TKFS 11111	1162	49	22	1137.90
20.	TKFS 1198	1248	19	5	851.90
21.	SPV 2123	1187	40	7	1194.33
22.	IS 18758	1484	23	9	699.47
23.	IS 18551	1627	38	16	733.60
24.	TAM 428	1620	40	11	768.10
25.	**CO (FS) 29**	**833**	**37**	**7**	**1304.85**
	CV	**0.04**	**1.04**	**7.39**	**9.33**
	CD(.05)	**0.8687**	**0.6567**	**1.2286**	**99.3176**
	SEd	**0.4320**	**0.3266**	**0.6110**	**50.0344**

The cyanogenic glycoside dhurrin present in sorghum makes a major limitation for its utilization as fodder. The safe limit of HCN in fresh fodder for livestock is 500 ppm (McBee and Miller,1980). At the age of 50th day, by which most of the entries started flowering, the level of HCN in most entries was evidently low and four entries recorded nil/zero level. The data indicated that HCN content of all 24 sorghum fodder accessions at maturity were found under safe limit of 500 ppm for livestock. The results of trend analysis made on 35th day indicated that the values of HCN-p ranged from 9 ppm in TNS 623 to 109 ppm in K11. Hence, it is inferred that this reference set could be used for feeding livestock from 35th day onwards.

Green fodder yield of *Sorghum* accessions with safe level of HCN-p: It has been observed that several high-yielding cultivars are not safe as fodder (Chaturvedi *et al.*, 1994). Among the accessions studied four lines *viz*., TKFS 11107, TNS

623, TKFS 1051 and TKSV 1046 recorded zero/nil HCN on 50th day with GFY/plant of 887.1g, 575.27g, 1117.07g and 437.67g respectively.

With respect to GFY/plant, K3 and TKSV 1050 recorded significantly higher biomass as compared to the check CO (FS) 29. These entries could be concentrated further for utilization in hybridization programme to develop sorghum varieties with high fodder yield and quality.

Path analysis: Path analysis carried out for nine fodder yielding traits along with the Cyanogenic potential (HCN-p) using GENRES software showed that HCN-p had the highest contribution to the diversity (85.87%). This justifies that the reference set thus developed in this study represents the value of cyanogenic potential (HCN-p).

Conclusion

It is concluded from this study that FAD method could be used for rapid screening of large scale sorghum accessions for HCN-p. The reference set developed from this study could well be utilized further in breeding programmes to evolve sorghum genotypes possessing higher fodder yield and low HCN content.

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