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Estimation of HCN content in sorghum under irrigated and stressed conditions

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Abstract
Premise of research: Sorghum (*Sorghum bicolor* (L.) Monech) is one of the most important fodder crops for ruminants and it is a dual purpose crop used as a food and fodder but one of the great limiting factor with the forage sorghum is that it is having an anti-nutritional factor cyanogenesis, which is extremely toxic to the animals when feeding on the livestock. Sorghum leaf and stem tissues produces dhurrin, during the early growth stages; after a drought or heat stress or after a cutting for fodder etc. The effect of cyanogenic (HCN-producing) glycoside dhurrin (β-D-glucopyrinosyloxy-(S)-p-hydroxy-mandelontrile) which lowers the nutritive value of sorghum fodder. Hence, there is a need to improve the forage quality and also identify the sorghum genotype which will produce the safe levels of HCN that would be used as a livestock for animal feeding. Therefore, this present was designed to study/predict the amount of HCN released in both the irrigated and stressed (drought) conditions in sorghum plants.
Pivotal results: To identify the cyanogenesis and to predict the exact stage where cyanogenesis is maximum in sorghum fodder, 5 prominent sorghum genotypes viz. EJ24, SSG59-3, SPV-462, CSV-15, IS18845 are used for HCN estimation in multi-cut hybrid and single cult hybrids. The cyanogenesis of these genotypes was determined by estimating the HCN in the intervals of 15, 30, 45, 60 and 80 days after sowing of sorghum plants. The results of the cyanogenesis analysed after 45 days of sowing showed that the high levels of HCN released in all five genotypes of sorghum under stressed (drought) conditions when compared with the irrigated conditions.
Conclusions: Based on the obtained results it was concluded that hydrogen cyanide (HCN) production was more severe in the plants grown under drought conditions when compared with plants grown under irrigated conditions. It was also noticed that forage sorghum genotypes exhibited greater cyanogenic toxicity especially if fed in early growth stages. Increased uptake of forage sorghum grown under irrigated conditions is required such that farmers are assured of cyanogen-safe fodder. From these studies it was recommended that sorghum crop cultivated especially under drought conditions should be strictly evaluated for HCN estimation before its use as fodder for livestock.

Keywords: triclosan, TCS, determination, detection, sensor

Introduction
Sorghum (*Sorghum bicolor* (L.) Monech) belongs to a grass family poaceae. Sorghum is having commercial importance worldwide. Sorghum is an important food crop also used as a fodder for animals, bio-fuel, and alcohol beverages production. Sorghum is the fifth important cereal crop, grows under drought and heat-tolerant conditions. Sorghum has substantial popularity amongst farmers in due to its greater adaptability and various forms of utilization like green fodder, Stover, silage and hay to suit the diverse needs of farming systems, besides its grain. Sorghum fodder constitutes 20-45 per cent of the total dry weight of feed of dairy animals during rainy season and up to 60 per cent during the periods of summer and winter, in India. Therefore, the need for genetic improvement of sorghum for increased yields and nutritive value of fodder is having high priority. One of the major anti-quality factors of sorghum fodder is the cyanogenic (HCN-producing) glycoside dhurrin that lowers the nutritive value due to its toxic effects on the feeding livestock. Dhurrin is produced in sorghum leaf and stem tissues, especially in early growth stages, after a drought or heat stress, after a cutting (for fodder), etc. The dhurrin is hydrolyzed in the rumen to produce the toxic HCN. Prussic acid causes death of animals by interfering with the ability of red corpuscles in the blood to transfer oxygen.
The quantity of dhurrin content in sorghum leaves and stem varies depending on genotype, plant age and growth conditions. Dhurrin accumulates in sorghum tissues during rapid growth following periods when environmental conditions are unfavourable for growth. Thus, in case of multi-cut forage sorghum, rapidly growing new shoots after a rain that terminated a drought, or frost, has very high HCN potential. This potential is genetically regulated, so that different cultivars can have very different HCN potential values under similar circumstances.

The present study is about the HCN estimation in multi-cut hybrid and single cult hybrids in sorghum plants and determination of the stage in which cyanogenesis is maximum in sorghum fodder. The genotypes EJ24, SSG59-3, SPV-462, CSV-15, IS18845 (in pots) in 2 replications with 4 plants per each replication mainly used for this study.

Material and Methods

Plant material

The sorghum genotypes viz., EJ 24, SSG59-3, SPV 462, CSV 15 and IS18845 were raised in pots in 2 replications with 4 plants per each replication in glasshouse and were used for the cyanogenic analysis. The plants were allowed to grow up to 3 months and both irrigated and stressed conditions were maintained. In between the 3 months plant leaf samples were collected freshly at the intervals of 15, 30, 45, 60, and 80 days for the cyanogenesis analysis after sowing. The HCN content was estimated as per the picric acid method suggested by Hogg and Ahlgren (1942) [4] and expressed in parts per million (ppm) on dry weight. In each row among all plants three representative plants were selected. Collected sorghum plants neatly cleaned insect, and dust free sorghum leaf sample used. Plant dried on filter paper for some time to remove the moisture. The selected part of healthy leaf and stem chopped in to very fine pieces. From the chopped plant material 1 gram weighed and transferred to the 15ml glass test tube to this 200µl of chloroform added. Filter paper strips dipped in to the picric acid solution having specific diameter were hang into the test tube with the help of cork. Test tubes Incubated for 24 h at room temperature. After 24 h the filter paper strips turned to reddish-brown colour is an indication of HCN release. The filter paper strips were transfer to 10 ml distilled water in another set of 15 ml test tubes. By using the vortex mixer, filter paper stripes were mixed properly to get complete transfer of the reddish-brown colour in to water. Using a spectrophotometer the OD value of samples measured at 515 nm length. The values from the spectrophotometer reading standard graph were compared with the standard curve prepared with the help of KCN.

Results

Depending on the plant age and growth conditions the amount of cyanogenic glucoside durrin shows variation in sorghum (*Sorghum bicolor* (L.) Monech) plants (PK Busk, BL Moller, 2002) [2]. The present investigation was conducted to determine the stage at which cyanogenesis is maximum in sorghum fodder and effect of drought stress in five genotypes of sorghum.

Cyanogenesis in five genotypes were determined at 15, 30, 45, 60 and 80 days after sowing (in pots) in 2 replications with 4 plants per each replication (Table: 1 & Fig: 1). The picric acid method was used for bioassay from leaf and stem portions. The HCN levels were higher till 45-60 days in all genotypes tested and decreased thereafter. The genotypes EJ24, SSG59-3, SPV-462, CSV-15, IS18845 (all *Sorghum bicolor*) and IS 18845 (*Sorghum halepense*) exhibited HCN levels above threshold limits of 200 ppm before flowering. The forage variety SSG 59-3 (*S. bicolor* x *S. sudanense*) exhibited safe levels of HCN throughout the period tested. Higher levels of HCN were observed at 45 DAS (Days after sowing) in all genotypes of *S. bicolor* (Table: 1). Cyanogenesis in these fourteen genotypes were determined at 45 days after sowing (in pots) in 2 replications with 4 plants per each replication, as above in plants with irrigation (to field capacity everyday) and drought stress (irrigation stopped 3 days before HCN estimation, i.e., on 42th day after sowing). The HCN levels increased with drought stress in all genotypes tested (Table: 2 & Fig: 2).

Table 1: HCN levels in forage lines at different growth stages

S. No.	Genotype Name	Days after sowing				
		15	30	45	60	80
1	EJ24	170.58	255.91	193.78	202.3	45.9
2	SSG59-3	3.59	188.56	133.6	118.8	66.8
3	SPV-462	10.7	117.1	144.5	179.5	36.8
4	CSV-15	6.5	114.01	347.2	324.1	35.4
5	IS18845	281	307.2	389.6	424.5	164.7

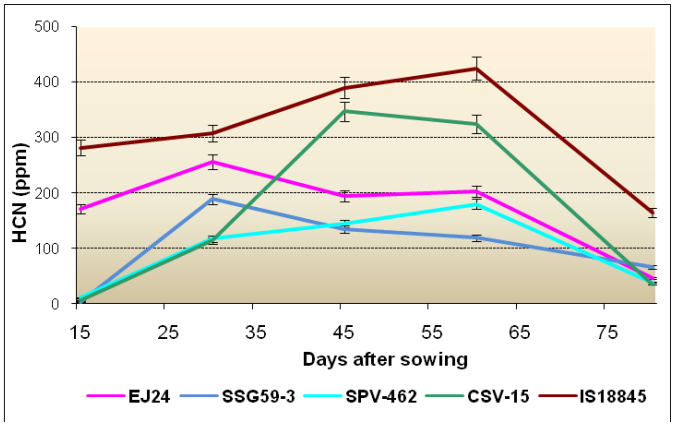

Fig 1: HCN content variation among genotypes along with plant growth period

Table 2: HCN levels in Irrigated and Stressed conditions

Genotype	Irrigated	Stressed	% increase in HCN in stress
EJ24	131.2	256.3	95.4
SSG59-3	101.5	165.6	63.2
SPV-462	123.4	165.6	34.2
CSV-15	249	445.2	78.8
IS18845	389.6	455.4	16.9

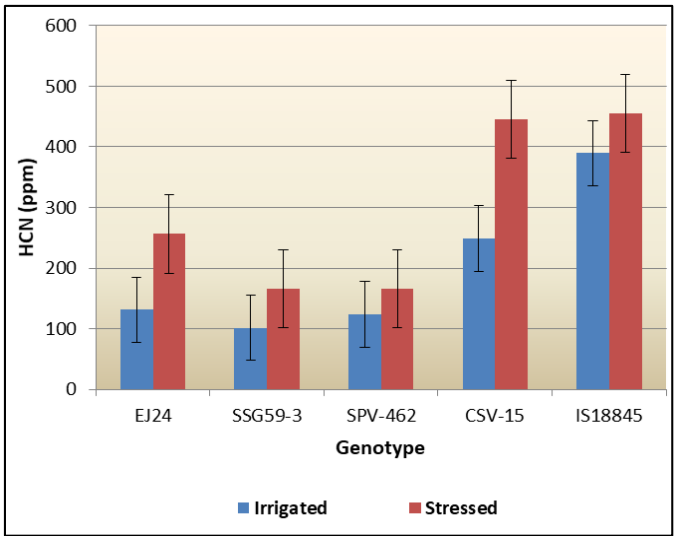

Fig 2: HCN levels in sorghum genotypes at 45 Days after sowing with irrigation and stress (Irrigation withheld at 42 days after sowing)

The per cent increase in HCN content was highest in EJ 24 (95.4%), followed by CSV 15 (78.8%) and SSG 59-3 (63.2%). The increase in HCN content of *S. halepense* genotype IS 18845 was minimal (16.9%), compared to *S. bicolor* genotypes. This may be owing to greater levels of

HCN already present in the non-stressed plants of *S. halepense*. These findings indicate that screening of sorghum genotypes may be more reliably done under drought stress to identify genotypes that possess lower cyanogenesis even under stress conditions. All lines possessed HCN levels below the thresh hold of 200 ppm, they are safe for feeding to cattle as green forage under normal cultivation.

Discussion

The main objective in the present study is about the estimation of cyanogenesis in sorghum plants under irrigated and stressed conditions. When compared to the irrigated conditions stressed condition showed high levels of HCN. Forage sorghum genotypes were often associated with cyanogenic toxicity especially if fed in early growth stages. Whereas the forage sorghum cultivars produce the highest DDM/day under conditions with minimal irrigation. Increased uptake of forage sorghum required that farmers are assured of cyanogen-safe fodder. Cyanogenesis, *i.e.*, production of HCN in forage sorghum, is a problem for feeding younger sorghum plants to cattle. There has been emphasis on using low HCN sorghum genotypes for forage sorghum breeding for both single and multi-cut purposes.

Hydrocyanic acid (HCN) is an antinutritional factor which is potentially toxic to the animal when fed on 30–35-day-old sorghum crops (Wheeler *et al*. 1990) [7]; HCN content <200 mg/g on dry-weight basis is safe for animal consumption. Content of HCN was estimated on green plant samples (0.20 g of minced tissue) sampled at 35 days after sowing. Estimation of HCN was performed according to the procedure of Gilchrist *et al*. (1967) [3]. HCN content in sorghum fodder is high when it grown in soil rich with nitrogen and phosphorous (Pandey RK *et al*; 2011) [6]. Sorghum is high yield producing crop and also popularly utilised as forage crop (Ouda JO *et al*; 2001) [5]. Sorghum cultivars during rain-fed regions produce lowest level of HCN and at pre-booting stage is safer for livestock feeding (Zahid AD *et al*; 2012) [8]. Mutant sorghum plants produce less or very low dhurrin content because of these reason mutants suits for forage production (Blomstedt CK *et al*; 2012) [1].

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