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Hydrocyanic acid, fodder quality and yield variation in sorghum genotypes grown under varying fertilizer levels

Harpreet Kaur Oberoi[1*], Maninder Kaur[1] and R. K. Agrawal[2]
[1]Punjab Agricultural University, Ludhiana-141004, India
[2]ICAR-Indian Grassland and Fodder Research Institute, Jhansi-284003, India
[*]Corresponding author e-mail: harpreetoberoi@pau.edu
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Abstract
Sorghum (*Sorghum bicolor* (L.) Moench) is grown as *Kharif* crop in dry land farming and provides fodder for livestock. However, this crop produces hydrocyanic acid (HCN) toxin that if ingested in higher amount could be fatal to animals. The HCN content in sorghum varies depending on plant age, fertilization and cultivar. Keeping this in view, an experiment was conducted to evaluate variability of HCN content in fresh leaves of fodder sorghum genotypes *viz.,* SPV 2445, CSV 21F and CSV 30F grown under varying fertilizer levels at different growth stages. Variation in crude protein content and *in vitro* dry matter digestibility in sorghum genotypes as influenced by fertilization when harvested at 50% flowering stage was also investigated. The fodder sorghum genotypes were sown under three fertilization levels (75, 100 and 125% recommended dose of fertilizer). Study showed that genotypes and fertilizer levels had a variable influence on quality of sorghum. The sorghum genotype CSV 21F in comparison to other two genotypes accumulated lesser HCN content during development at 125% RDF. The genotype CSV 21 F was also superior in CP and IVDMD in mixture followed by leaves alone when harvested at 50% flowering stage. It was concluded that sorghum genotype CSV 21F with 125% RDF application produced better quality fodder.

Keywords: Fertilizer levels, Fodder quality, Growth stage, Hydrocyanic acid, Sorghum genotypes

Introduction
Forage is a basic feed resource for ruminant animals. Accordingly knowledge on forage quality is required by farmers so that they can select the best quality forage offered to their livestock. Forage quality is directly related to the extent to which the plant provides nutrient to animals (Mahyuddin, 2008). Sorghum (*Sorghum bicolor* (L.) Moench) is one of the important summer fodder crops all over the country particularly in rainfed regions having suitability to wide variation in soil and climatic conditions and other advantages like quick growth, high biomass accumulation, high dry matter content and wide adaptability. For providing, nutritious, juicy, palatable fodder, it is well-liked by the cattle. Because of its high tolerance to various stresses, it is extensively grown as a major source of fodder and preferred over maize (Reddy *et al*., 2004). It is also suitable for silage and hay making.

No forage production system seems to be complete without selection of suitable varieties and fertilization, which influence plant characteristics. Farmers use these practices to improve the quality of forage, in addition to enhance or delay cut of forage. Among the nutrients, nitrogen is vital for plant growth and is the most limiting nutrient in Indian soils. It promotes shoot elongation, tillering regeneration, leaf to stem ratio, succulence and palatability of fodder crop (Karthika and Kalpana, 2017). Nitrogen application is essential requisite to utilize the available soil and environmental resources effectively. Nitrogen application directly influences the quality of the forage (Kaur *et al.,* 2016). The increase in nitrogen fertilization improved considerably nitrogen uptake, contributing to large photosynthetic activity and synthesis of protein. Forages with high crude protein (CP) are considered of high quality and must be included in the diets of livestock for their proper growth and development (Kaur and Goyal, 2017). Moreover, they also cut down the need of supplementary protein. Farmers are generally not familiar with the growth stage of forage sorghum that should be fed to the livestock. They apply nitrogen fertilizer to get higher forage yield of sorghum and feed to livestock at any growth stage without having the knowledge of HCN (Dhurrin) poisoning, which in turn affects the animal health (Aziz-Abdel and Abdel-Gwad, 2008). Exposure to HCN can lead mild to severe intoxication while in extreme cases leads to death of animals which might be due to it inhibition of cytochrome oxidase, an enzyme of electron transport chain (Leavesley *et al.,* 2008). HCN contents vary in different plant parts of sorghum, being high in leaves as compared to stems (Nahrstedt, 1993; Vetter, 2000). The overall HCN concentration in plant tissue decreases with increase in stem weight (Busk and Moller, 2002). Different varieties of sorghum develop variable levels of HCN when grown under different environmental conditions (Khatri *et al*., 1997). Hence, this study was formulated to evaluate the effect of varying fertilizer levels on HCN content, other fodder quality parameters *viz*., crude protein (CP), *in vitro* dry matter digestibility (IVDMD) and yield in three single cut forage sorghum genotypes.

Materials and Methods

***Experimental location, climatic data and soil features*:** A field experiment was conducted during *Kharif* season of the year 2018 at Forage Research Farm of Punjab Agricultural University, Ludhiana, India. The study area is situated at 30° 54' N latitude and 75° 48' E longitude with an altitude of 247 meters above the mean sea level. Further, the experimental site has semi-arid and sub-tropical climate with hot dry summer and severe cold winter. The soil of the experimental field was sandy loam in texture with pH 7.8, available nitrogen 184 kg/ha, available phosphorus 16.8 kg/ha and available potassium 246 kg/ha.

***Experimental set-up and crop management*:** The experiment was consisted of 9 treatment combinations comprising three single-cut forage sorghum genotypes (SPV 2445, CSV 21 F and CSV 30 F) sown in opened furrows at 25 cm apart using seed rate of 40 kg/ha and three fertilization levels *viz.,* 75, 100 and 125 per cent of recommended dose of fertilizer (RDF). These treatments were tested in factorial randomized block design with three replications. The recommended dose of fertilizer (100%) for forage sorghum is 100 kg N, 20 kg P_2O_5 and 25 kg K_2O (in potassium deficient soils) per hectare. Half dose of nitrogen and full dose of phosphorus and potassium were applied as basal according to treatment levels. Remaining half dose of nitrogen was top-dressed after first irrigation, 30 days after sowing (DAS). The crop received uniform irrigation as per requirement. All other standard agronomic practices for the cultivation of forage sorghum were followed uniformly in all the treatments.

***Observations*:** The HCN content was estimated in fresh samples at different stages of growth (*i.e.* 50 DAS, 60 DAS, pre-booting, booting and 50% flowering stage) by following the method of Hogg and Ahlgren (1942). The crop was harvested at 50% flowering stage and the plant samples were taken for CP and IVDMD analysis in stem, leaves and mixture. Plant samples collected after harvest were sun dried and then completely dried in hot air oven till a constant weight was obtained. The dried samples were ground using Willy grinder mill to a uniform mesh size and used for the estimation of crude protein (CP) and *in-vitro* dry matter digestibility (IVDMD) according to the method of AOAC (1970) and Tilley and Terry (1963), respectively. Yield and yield related parameters were recorded to find correlation with fodder quality traits.

***Statistical analysis*:** Data on forage yield, HCN content, *in vitro* dry matter digestibility (IVDMD) and crude protein (CP) content were analyzed by using OPSTAT software available on CCS Haryana Agricultural University website (Sheoran *et al.,* 1998). The results were presented at five per cent level of significance (P=0.05) for making comparison between treatments.

Results and Discussion

***Green and dry fodder yield*:** Different sorghum genotypes tested varied significantly among themselves for green fodder and dry matter yields. The data revealed that genotype CSV 21 F recorded significantly highest green fodder (63.30 t/ha) and dry matter (16.20 t/ha) yields as compared to the genotypes (Table 1). This could be attributed to higher number of tillers and plant height of CSV 21 F. Several workers also observed variation among the sorghum varieties for forage and ancillary characters (Kaur *et al.,* 2018; Kaur and Satpal 2019; Shivaprasad *et al.,* 2019). The variation in varieties could be due to the differences in their genetic makeup. The differential behavior of these genotypes could also be explained solely by variation in their genetic makeup (Meena *et al*., 2012). Green fodder and dry matter yield of sorghum increased with successive increase in fertility level. The application of 125% of RDF produced 7.30 and 20.10% higher green fodder and 9.70 and 10.40% higher dry matter over 100 and 75% RDF, respectively. For green and dry fodder yield, the interactive effects between fertilizer levels and genotypes were observed non-significant (Table 1). This could be due to the increased availability and absorption of nitrogen at higher doses which resulted in increased vegetative growth. The similar increase in green fodder and dry matter yields for different sorghum genotypes were also reported by Satpal *et al*. (2015). Shanti *et al.* (2019) reported the significant response in green fodder yield, dry matter yield and protein content in sorghum genotypes with increasing fertilizer levels. Patil *et al.* (2018) observed significantly higher dry fodder yield in perennial fodder sorghum cv. CoFS-29 sown with the highest fertilizer level.

Table 1. Green fodder and dry matter yields of sorghum as influenced by genotypes and fertilizer levels at 50% flowering stage

Treatments	Green fodder yield (t/ha)	Dry matter yield (t/ha)
Genotypes		
SPV 2445	57.20[b]	13.90[b]
CSV 21 F	63.30[a]	16.20[a]
CSV 30 F	51.20[c]	14.70[b]
CD (P<0.05)	5.30	1.30
Fertilizer levels		
75% RDF	51.70[b]	14.40[b]
100% RDF	57.90[a]	14.50[b]
125% RDF	62.10[a]	15.90[a]
CD (P<0.05)	5.30	1.30
G×F	9.10[ns]	2.20[ns]

The letters in the same column represent significant difference at P<0.05, and the same letters indicate non-significant difference; NS: Non-significant

***HCN content*:** Sorghum may be harmful for livestock under certain conditions *i.e.,* if fertility of soil, plant growth stage and weather conditions that inhibit plant growth, which in turn enhanced cyanogenic glucosides production (Ramos *et al.*, 1998; Wheeler, 1994). The maximum HCN content of 78, 84, 68, 43 and 22 ppm was recorded in CSV 30F at 50 DAS, 60 DAS, pre-booting, booting and 50% flowering stage, respectively (Table 2). Sorghum genotype CSV 21F yielded 13, 3, 3, 9 and 7% less HCN over SPV 2445 sorghum genotype and 30, 14, 16, 21 and 20% less HCN content over CSV 30F at 50 DAS, 60 DAS, pre-booting, booting and 50% flowering stage, respectively. The genetic makeup of the sorghum genotypes might be responsible for the above observed difference in HCN production throughout their growth period and similar observation was reported earlier by Hanuman *et al*. (2008) in sorghum cultivars. Further, Gorz *et al*. (1987) stated that the growth conditions and the genetic background highly affected the cyanide quantity in both young and mature plants. In the present study, CSV 30F genotype produced more HCN as compared to SPV 2445 and CSV 21F sorghum genotypes. The HCN contents recorded in our experiment were many-folds less (16 to 84 ppm) in different sorghum cultivars as compared to those reported by Moaveni (2010), who estimated HCN content ranged between 216 and 243 ppm. While Sarfraz *et al*. (2012) observed that HCN content ranged between 255 and 347 ppm in different sorghum varieties. The difference in values might be due to various genetic characters and stem thickness of tested genotypes.

In the present study, the increase in RDF levels showed decrease in HCN production (Table 3) and this might be due to thick canopy, resulting in reduction in temperature and increase in humidity beneath the foliage and ultimately affecting quality by lowering HCN content. It was reported that the HCN proportion decreases as the height of the plant increases due to which it is harmful for the livestock at initial stages because of presence of more cyanide and toxicity decreases with maturity of plant (Pistoia *et al.,* 2003). Sher *et al*. (2016) also reported that high nitrogen application resulted in good vegetative growth and dense canopy of plants thereby, affecting forage sorghum quality. The HCN content with higher values was recorded at 60 DAS in all the three sorghum genotypes and at different levels of nitrogen application. The HCN content in sorghum genotypes were higher at early stages of maturity, however, with the advancement

Table 2. Hydrocyanic acid (ppm) content at different growth stages of sorghum as influenced by genotypes and fertilizer levels

Treatments	50 DAS	60 DAS	Pre-Booting	Booting	50% Flowering Stage
Genotypes					
SPV 2445	62.40[b]	74.50[b]	58.90[b]	37.10[b]	23.50[a]
CSV 21 F	54.50[c]	72.40[c]	57.10[b]	33.70[c]	17.60[c]
CSV 30 F	77.90[a]	84.10[a]	68.20[a]	42.60[a]	21.90[b]
CD (P<0.05)	2.80	1.50	2.80	2.60	1.60
Fertilizer levels					
75% RDF	75.40[a]	88.80[a]	71.20[a]	39.10[a]	16.20[b]
100% RDF	65.60[b]	75.90[b]	60.60[b]	36.70[a]	23.40[a]
125% RDF	53.90[c]	66.90[c]	52.50[c]	37.50[a]	23.40[a]
CD (P<0.05)	2.80	1.50	2.80	NS	1.60
G×F	4.90	2.50	NS	4.50	2.80

The letters in the same column represent significant difference at P<0.05, and the same letters indicate non-significant difference; NS: Non-significant in the maturity the HCN content was decreased. The minimum HCN content was recorded at 50% flowering stage with different levels of fertilizer application. Interactive effect of genotype and fertilizer level showed significant variation at all stages except at pre-booting stage. From the above results, it could be suggested that the genotypic difference among the sorghum genotypes was there, which probably resulted in different HCN accumulation at different stages of growth.

***Quality parameters*:** The forage digestibility is related to change in chemical composition and to some extent to the protein content (Sher *et al.,* 2014). Therefore, forage containing high crude protein content is considered of good quality. Rahmani *et al.* (2008) reported that nitrogen is the most important element in protein synthesis and its increase in optimum conditions increases the amount of protein. Crude protein plays an important role in fodder quality as it is required for lactation, growth and reproduction of ruminant animals. The present investigation on crude protein (CP) content and *in vitro* dry matter digestibility (IVDMD) variation in different plant portions of three forage genotypes showed significantly higher crude protein at 125% of RDF application over 75 and 100% of RDF in stem, leaves and mixture of sorghum plant tissues (Table 3). Similarly, *in vitro* dry matter digestibility was higher with 125% of RDF but non-significant variation in IVDMD was observed in stem and mixture sorghum samples in comparison to leaves (Table 4). The application of different fertilizer levels was found to have direct relationship with crude protein content of sorghum genotypes (Table 4). The maximum CP contents of 5.9, 7.5 and 7.8% were recorded in stem, leaves and mixture portion, respectively, in plots where nitrogen was applied at 125% RDF and the lower CP contents of 5.2, 6.4 and 7.0% were recorded at 75% RDF, respectively, in three different plant portions i.e. stem, leaves and mixture. Verma *et al.* (2005) also observed highest CP content and digestibility of sorghum fodder at 120 kg N/ha than 30, 60 and 90 kg N/ha. The increase in protein content with increase in nitrogen level might be due to enhanced nitrogen for amino acid formation, which resulted increase in protein content.

The crude protein content and IVDMD as fodder quality components under study significantly varied among the three different sorghum genotypes. The genotypic variations might have influenced the accumulations of these fodder quality traits. The genotypes CSV 21F and CSV 30F showed maximum CP content compared to the genotype SPV 2445 in the three different plant portions *i.e*. stem, leaves and mixture. The interactive effects of genotype and fertilizer exhibited significant differences for CP in stem and leaves plant parts and non-significant difference was observed in mixture (Table 3). The higher *in vitro* dry matter digestibility *i.e*. 50.40, 42.60 and 58.30% were observed in CSV 21F stem, leaves and mixture plant portion, respectively (Table 4). The improvement in quality might be due to the fact that nitrogen being as essential constituent of chlorophyll, protoplasm, protein and nucleic acids are needed for protein synthesis. As such adequate available nitrogen enhanced the protein synthesis, which resulted in higher content as well as yield of crude protein. Interactive effects of genotype and fertilizer for IVDMD revealed non-significant variation in stem and leaves of all three sorghum genotypes (Table 4). The contents of CP and IVDMD were higher in the mixture among all the plant portions of three forage sorghum genotypes (Tables 3-4). Further, the stem portion showed lower CP in comparison to leaves in all three genotypes. On the contrary, IVDMD of stem was recorded as higher than that of leaves.

Table 3. Crude protein content (%) in different plant tissues (stem, leaves and mixture) of sorghum genotypes as influenced by genotypes and fertilizer levels at 50% flowering stage

Treatments	Stem	Leaves	Mixture
Genotypes			
SPV 2445	5.40^{b}	6.70^{b}	7.30^{b}
CSV 21 F	5.90^{a}	7.30^{a}	7.60^{a}
CSV 30 F	5.70^{a}	7.10^{a}	7.40^{b}
CD (P<0.05)	0.30	0.20	0.10
Fertilizer levels			
75% RDF	5.20^{c}	6.40^{c}	7.00^{c}
100% RDF	5.70^{b}	7.20^{b}	7.50^{b}
125% RDF	5.90^{a}	7.50^{a}	7.80^{a}
CD (P<0.05)	0.30	0.20	0.10
G×F	0.50	NS	0.20

The letters in the same column represent significant difference at P<0.05, and the same letters indicate non-significant difference; NS: Non-significant

The forage sorghum genotypes in the current study showed variation in crude protein content and thereby, indicated the genotypic effect on their proximate composition. Genotype CSV 21F appeared more nutritious for animals as it contained significantly higher crude protein which was more than 7% in both leaves and mixture portions in comparison with other two genotypes. Aruna *et al.* (2015) reported significant differences among the sorghum cultivars for crude protein contents. Protein is one of the costliest supple-

-ments for livestock and the total amount of protein produced per unit area is one of the most important quality characteristics in forage based feeding of animals (Lithourgidis *et al*., 2006).

Table 4. *In vitro* dry matter digestibility (%) in different plant tissues (stem, leaves and mixture) of sorghum genotypes as influenced by genotypes and fertilizer levels at 50% flowering stage

Treatments	Stem	Leaves	Mixture
Genotypes			
SPV 2445	43.90[b]	37.00[b]	48.3[b]
CSV 21 F	50.40[a]	42.60[a]	58.3[a]
CSV 30 F	44.50[b]	38.40[b]	50.20[b]
CD (P<0.05)	3.90	1.40	2.90
Fertilizer levels			
75% RDF	45.20[a]	39.10[b]	50.60[b]
100% RDF	45.50[a]	40.70[a]	52.90[a]
125% RDF	48.10[a]	38.10[b]	53.20[a]
CD (P<0.05)	NS	1.40	NS
G×F	NS	2.50	NS

The letters in the same column represent significant difference at P<0.05, and the same letters indicate non-significant difference; NS: Non-significant

Conclusion

The sorghum genotype CSV 21F was found superior with higher fodder quality characters *i.e*. CP and IVDMD and produced less HCN. Therefore, it was concluded that adoption of the superior cultivars, management strategies like harvest at 50% flowering stage and use of mixture of plant parts will help to obtain better quality forage sorghum with reduced HCN to feed the livestock.

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