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HYDROCYANIC ACID AND SUGAR CONTENT DYNAMICS UNDER NITROGEN AND SULPHUR APPLICATION TO FORAGE SORGHUM CULTIVARS

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ABSTRACT

Sorghum crop provides excellent forage in dry land areas. It has ability to tolerate high temperature and grow under minimal soil moisture. Sugar content in Sorghum is of prime importance regarding, animal feed value as well as biofuel production. However, high level of HCN toxin production is a serious problem associated with this crop which could be lethal to animals if ingested in greater quantities. In this study hydrocyanic acid and sugar content dynamics were determined on two forage sorghum cultivars i.e. JS-2002 and Chakwal sorghum at different developmental stages (booting[a] and 50% heading[b]). Fertilizer treatments of nitrogen (0, 60 and 120 kg ha^{-1}) and sulphur levels (0, 20 and 40 kg ha^{-1}) were applied in replicates. The experiment was arranged in randomized complete block design. The results clearly depicted that increase in nitrogen levels gradually increased the HCN and sugar contents irrespective to sulphur and delayed harvesting times. Increase in HCN (21[a] & 22[b]%) and brix value (56[a] & 59[b]%) was found more at 120 kg N ha^{-1} when compared with 0 kg N ha^{-1} (control) treatment. Similarly increase in sulphur application caused increase in brix value however; inverse relationship with HCN content was recorded. Results also indicated that sorghum cultivar JS-2002 produced 27[a] & 40[b]% decline in HCN content while 8[a] & 18[b]% more sugar content when compared with Chakwal sorghum. Seasonal variations resulted maximum sugar production of 14.9, 25.6% and HCN content of 21.9 & 13.1 mg/100g during different year time (2010 and 2009 when compared with 2008, respectively) probably due to variation in seasonal rainfall. Three years field study indicate that JS-2002 produced less HCN with low dose of N application (0 kg ha^{-1}) and higher application of sulphur (40 kg ha^{-1}) while more brix value at 40 kg S and 120 kg N ha^{-1} under sub-tropical rainfed conditions.

Keywords: Brix value, HCN, nitrogen, sorghum cultivars, sub-tropical condition, sulphur

INTRODUCTION

Sorghum is one of the important summer fodder crops all over the country particularly in rainfed regions. It is nutritious, juicy, palatable and well-liked by the cattle. Because of its high tolerance to various stresses, it is extensively grown as a major source of fodder preferred over maize (Reddy *et al.,* 2004). Sorghum plants contain a cyanogenic glycoside called dhurrin. According to Lang (2001) when the plants cells damaged (insect attack, crust, hailing, frost injury), the available cyanogenic glycoside (dhurrin) is hydrolyzed by making two fermentation enzymes (*Ac/ac and Li/li*) which converted into hydrocyanic acid (HCN). Cyanogenesis is a character which genetically controlled, but it is often affected by different biotic and abiotic factors (Pederson *et al.,* 1996). The main factors responsible for HCN accumulation are plant species (Sudan grass have less HCN contents than sorghum), plant organs (leaves have higher HCN contents than other parts), suckers (tillers have more HCN potential), fertilizer (unevenness nitrogen and phosphorus in the soil causes the HCN contents to increase) and drought (dryland sorghum has more HCN contents than irrigated one due to water stress).

The available amount of cyanide poisoning in forage is important to consider for animal's physiological conditions. On the wet weight basis if HCN level in forage sorghum exceed 200 mg kg^{-1} is harmful for livestock (Fjell *et al.,* 1991).

No doubt nitrogen application is considered essential for efficient plant growth and cell division (Duli *et al.,* 2005; Saraswathy *et al.,* 2007). However, higher level of nitrogen application may increase HCN contents of forage sorghum and thus poisoning animals (Aziz and Abdel-Gwad, 2008). Application of nitrogen fertilizer also increases brix value (Pholsen and Sornsungnoen, 2004). Different growth stages of sorghum produce varying levels of sugar in stem quantitatively and qualitatively specifically maximum sugar content are accumulated at maturity stage (Parvatikar and Manjunath, 1991). Nitrogen fertilizer promotes sucrose content, protein percent and growth rate in sweet sorghum (Tsialtas and Maslaris, 2005).

Sulphur is an essential element of nutrition for plant and animal whose deficiencies have been increasing throughout the world (Platou and Irish, 1982). The use of S-free mineral fertilizers has created widespread deficiency of Sin soils (Chaubey *et al.,* 1992). Sulphur fertilization can increase the yield and the quality of forages in the areas of S deficient soils (Puoli *et al.,* 1991; Hallmark and Brown, 1994). Moreover, S fertilization is known to improve nitrogen (N) utilization efficiency by the crops (Schnug and Haneklaus, 1993).

Most of the scientific work had focused on nitrogen but little attention had been paid to sulphur. Keeping in view this scenario, the present study was undertaken to explore the hydrocyanic acid and sugar content dynamics under nitrogen and sulphur application to forage sorghum cultivars sown under sub-tropical dryland conditions of Pakistan.

MATERIALS AND METHODS

Experiment was carried out at University Research Farm (URF) Chakwal Road (33° 56’ N, 72° 52’ E, 498 m a.s.l.) , PMAS- Arid Agriculture University Rawalpindi, during 2008, 2009 and 2010 to determine the hydrocyanic acid and sugar content dynamics with varying nitrogen and sulphur application to forage sorghum cultivars. The treatments consisted of two forage sorghum cultivars i.e. JS-2002 and Chakwal sorghum were sown with each three nitrogen levels (0, 60 and 120 kg ha^{-1}) in the form of urea and sulphur levels (0, 20 and 40 kg ha^{-1}) as gypsum arranged in randomized complete block design replicated four times.

Before sowing, soil sampling was carried out at two depths i.e. 0-15 and 15-30 cm from experimental site for physico-chemical properties. The soil was sandy-loam with pH about neutral. The total N content and organic matter were low, particularly in the lower layers of soil i.e. 15-30 cm. While phosphorus status (less than 5 mg P kg^{-1}) was observed (Olsen method): poor in both soil layers. Mean temperature (ºC), rainfall (mm) and relative humidity (%) data recorded during the study period is presented in Figure 1.

Determination of total cyanide through spectrophotometer

Leaf samples were chopped and ground with pestle and mortar. For calibration, a flat bottom plastic bottle with buffer of pH 6 and round filter paper disc were loaded. Grinded leaf sample (100 mg) was taken in the transparent bottle and poured 1 mL phosphate buffer solution in it. A yellow picrate paper was attached with the plastic stripe to absorb the HCN vapors and the upper end of stripe is further attached in the center of bottle lid with the help of tape and hanged in the

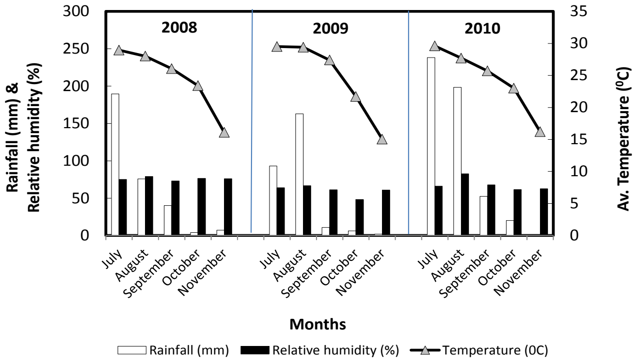

Figure 1. Rainfall, av. temperature and relative humidity recorded during crop growth period

bottle in such a way that picrate paper may not come in contact with liquid (leaf sample + buffer solution) lying in the bottom of bottle. A blank was also run having no leaves. Both the bottles were left for overnight at the room temperature. Next day the picrate paper was removed from plastic stripe cautiously. For about 30 minutes, the picrate paper was submerged into 5 mL distilled water with mild shaking. At 510 nm wavelength absorbance picrate solution was recorded by spectrophotometer as described by Bradbury *et al*. (1999). The hydrocyanic acid contents were calculated as under:

Total cyanide contents (mg kg^{-1}) =396 × absorbance reading.

Sweetness (%fresh stalk)

Sugar content (brix value) of forage sorghum was determined with the help of a refractometer (hand held brix refractometer, Sino Technology, Fujian, China), by the method of Yun-long *et al*. (2006).

Five plants were selected at random from each plot. The juice from the main stalk of each plant was extracted by simply twisting the stem. The extract of all five stalks was mixed and 0.5 ml of the mixed juice sample was applied to the automatic temperature compensating refractometer and the total sugar contents (as brix grades) were determined.

The HCN leaf content and sugar content were recorded at two growth stages viz. booting and 50 % heading.

The data collected were subjected to analysis of variance using the CoStat 6.3 software (CoHort Software, Monterey, CA, USA) to determine significance of the tested factors (cultivar, sulphur and nitrogen levels) and their interactions with sorghum traits. Means of significant effects were separated using the test at 5% level of significance.

RESULTS AND DISCUSSION

Hydrocyanic acid content

Hydrocyanic acid (HCN) content was observed in sorghum cultivars and results showed a significant difference (Table 1). The Chakwal sorghum produced the highest HCN content (22.6 and 15 mg/100 g) while the lowest was 11.5 and 9.0 mg/100 g by JS-2002 cultivar at booting and 50% heading stage, respectively. From the data, it appeared that 27-40% less HCN content was found in Js-2002 when compared with Chakwal sorghum at respective growth stages. The difference of HCN content may be due to the genetic makeup of different sorghum cultivars and climatic condition during the crop growth period at different years. The results are in agreement with findings of Hanuman *et al.* (2008) who reported that HCN content variation may depend on their genetic makeup of cultivars. Abusuwar and Hala (2010), Pandey *et al.* (2011), Sarfraz *et al.* (2012) and Sher *et al.* (2012) found higher HCN content in Abu Sabein, hybrid variety, Hegari and local sorghum, respectively than the other varieties. In our findings, Chakwal sorghum produced higher HCN content as compared with JS-2002. This decrease in HCN contents might be due to more green forage yield (data not shown) and sugar content.

Table 1. Interactive effect of Cultivar, Sulphur and Nitrogen application on hydrocyanic acid content (HCN) of sorghum forage in URF during summer 2008, 2009 and 2010

Treatments	**Growth stages**	
	Booting	**50% heading**
Cultivars (C)		
JS-2002	16.5 b	9.0 b
Chakwal sorghum	22.6 a	15.0 a
P	<0.001**	<0.001**
Sulphur Levels (S; Kg ha^{-1})		
0	28.4 a	17.1 a
20	17.9 b	11.0 b
40	12.3 c	8.0 c
P	<0.001**	<0.001*
Nitrogen Levels (N; Kg ha^{-1})		
0	17.8 c	11.0 c
60	19.3 b	11.7 b
120	21.5 a	13.4 a
P	<0.001**	<0.001**
Year (Y)		
2008	19.6 b	11.9 b
2009	21.9 a	13.1 a
2010	17.2 c	11.0 c
P	<0.001**	<0.001**
C × S		
P	1.00 ns	1.00 ns
C × N		
P	1.00 ns	1.00 ns
S × N		
P	<0.001**	<0.001**
C × S × N		
P	1.00 ns	1.00 ns
C × Y		
P	0.164 ns	0.748 ns
S × Y		
P	0.448 ns	0.605 ns
C × S × Y		
P	1.00 ns	1.00 ns
N × Y		
P	0.981 ns	0.995 ns
C × N × Y		
P	1.00 ns	1.00 ns
S × N × Y		
P	0.999 ns	0.992 ns
C × S × N × Y		
P	1.00 ns	1.00 ns
C.V. (%)	9.9	14.4

ns, *, ** mean non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively
Different letters indicate significantly-different means (SNK test; $P \leq 0.05$)

Regardless to cultivars, increase in sulphur levels reduced the HCN content. Minimum HCN content of 12.3 and 8.0 mg/100g was recorded with the application of 40 kg S ha^{-1} whereas maximum was 28.4 and 17.1 mg/100g in control plot at booting and 50% heading stage, respectively. The increase rate of sulphur resulted is 53-57% less HCN content in comparison with control plots. The reason might be the sulphur as an important component of chlorophyll, amino acids, enzymes and seed formation (Sharma, 1991).

With the increase in nitrogen fertilizer HCN content also gradually increased in sorghum plants (Table 1). Fertilizing sorghum forage with 120 kg N ha^{-1} produced the highest HCN content (21.5 and 13.4 mg/100g) at booting and 50% heading stage, respectively while the lowest HCN content of 17.8 and 11.0 mg/100g was recorded in control treatment where no fertilizer was applied. The increase of HCN content (21-22%) was more with the application of 120 kg N ha^{-1} when compared with control treatment (0 kg N ha^{-1}). No doubt N is an important component for plant growth and development (Shehu *et al.*, 2010). In sorghum plant increase in N level significantly increased HCN content which can be harmful, therefore it is important to explore the genetic source of sorghum which has less HCN trigger towards high N application. The similar results have been reported by Sher *et al.* (2012) that with the application of @ 120 kg N ha^{-1} produced 64% more HCN content than control plot. Bahrani and Ghenateghestani (2004) also reported an increase of 55% higher HCN content with nitrogen application in forage sorghum.

Seasonal variations produced minimum HCN content of 17.2 & 11.0 mg/100g during 2010 as compared to 2009 probably as result of higher seasonal rainfall. A reduction of 22 and 16% less HCN content was recorded during 2010 than 2009 at booting and 50% heading stage, respectively. In our experiments the HCN content does not exceed the safe limit during entire crop growth duration. The toxin (HCN) can exceed the safe limit (>200 mg kg^{-1}) if animals are fed for long time on the pre- mature stages and forage produced under low rainfall conditions (Sher *et al.*, 2012).

Significant interaction of sulphur and nitrogen application was observed at both growth stages (booting & 50% heading) during three years of study (Figure 2). Maximum HCN content of 31.9 and 19 mg/100g was recorded with the application of 120 kg N ha^{-1} and in control plots where no sulphur was applied at respective growth stages, respectively.

Brix value (%fresh stalk)

Brix value is an important characteristic to determine the forage quality. Significantly differences were recorded in the brix value of forage sorghum cultivars exposed to varying nitrogen and sulphur applications grown under subtropical rainfed regions during 2008 to 2010 (Table 2). Sorghum cultivar JS-2002 produced highest brix value of 13.3 and 23% whereas Chakwal sorghum resulted lowest brix value of 11.3 and 21.3% at booting and 50% heading stage, respectively. The results indicated 8 and 18% higher brix value in JS-2002 than Chakwal sorghum at booting and 50% heading stage, respectively. Mahammed and Moataz (2009) found a significant difference of brix value among different genotypes. Similarly, Almodares *et al.*, (2007) reported that the difference of brix value might be due to genetic variability and stem thickness. Present work indicated that sorghum cultivar JS-2002 produced highest brix value than Chakwal sorghum.

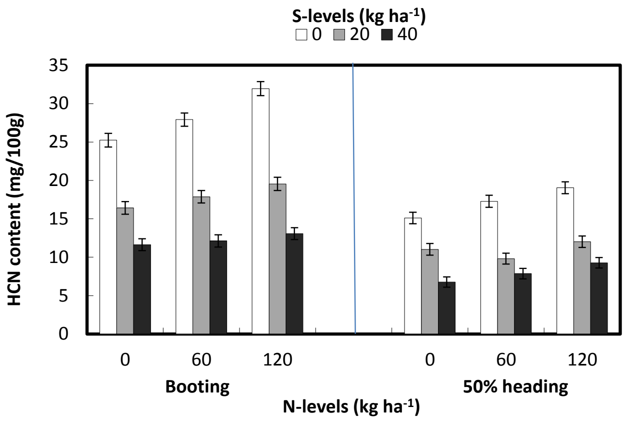

Figure 2. Effect of nitrogen and sulphur levels interaction on hydrocyanic acid content (HCN) at booting and 50% heading stage. Error bars represent the standard error (n = 5).

Increasing sulphur application progressively increased brix value of forage sorghum cultivars throughout the experimentation period under field conditions. Higher brix value of 13.3 and 23.8% was recorded with the application of 40 kg S ha^{-1} while lower (11.5 and 20.5%) in control treatment at respective growth stages, clearly indicating the positive affect of S application on forage sorghum. Regardless to sulphur, the highest brix value of 15 and 27.3% was observed with the application of 120 kg N ha^{-1}, which produced 56-59% higher brix value at booting and 50% heading stage, respectively than control treatment (9.6 and 17.2%) where no N was applied. Pholsen and Sukrsi (2007) found a range of brix value between 11 and 12.5% at N-K_2O rates. In our experiment, an average range of brix value (9.6 to 27.3%) was recorded at different nitrogen levels during study period. In contrast Almodares *et al.*, (2007, 2008) found no significant difference with the application of different nitrogen levels on brix value.

Seasonal variations produced maximum sugar content of 14.9, 25.6% at booting and 50% heading stage during 2010 as compared to 2009, respectively probably as result of seasonal rainfall variation as depicted in Figure 1.

Table 2. Interactive effect of Cultivar, Sulphur and Nitrogen application on brix value of sorghum forage in URF during summer 2008, 2009 and 2010

Treatments	**Growth stages**	
	Booting	**50% heading**
Cultivars (C)		
JS-2002	13.3 a	23.0 a
Chakwal sorghum	11.3 b	21.3 b
P	<0.001**	<0.001**
Sulphur Levels (S; Kg ha^{-1})		
0	11.5 c	20.5 c
20	12.1 b	22.2 b
40	13.2 a	23.8 a
P	<0.001**	<0.001*
Nitrogen Levels (N; Kg ha^{-1})		
0	9.6 c	17.2 c
60	12.1 b	22.0 b
120	15.0 a	27.3 a
P	<0.001**	<0.001**
Year (Y)		
2008	13.1 b	23.1 b
2009	8.8 c	17.7 c
2010	14.9 a	25.6 a
P	<0.001**	<0.001**
C × S		
P	0.800 ns	0.264 ns
C × N		
P	<0.046*	0.544 ns
S × N		
P	0.167 ns	0.106 ns
C × S × N		
P	0.616 ns	0.288 ns
C × Y		
P	<0.017*	<0.002*
S × Y		
P	<0.021*	<0.022*
C × S × Y		
P	0.717 ns	0.827 ns
N × Y		
P	<0.001**	<0.001**
C × N × Y		
P	0.672 ns	0.184 ns
S × N × Y		
P	0.370 ns	<0.047*
C × S × N × Y		
P	0.685 ns	0.968 ns
C.V. (%)	8.7	5.1

ns, *, ** mean non-significant, significant at $P \leq 0.05$ and $P \leq 0.01$, respectively
Different letters indicate significantly-different means (SNK test; $P \leq 0.05$)

Interaction between cultivar and nitrogen levels showed statistically significance difference in brix values at booting stage (Figure 3) while 50% heading stage remained non-significant. Maximum brix value 16.2% was recorded by JS-2002 with 120 kg ha^{-1} nitrogen fertilizer application while minimum was 8.8% in Chakwal Sorghum where no nitrogen was made. Significant interaction of C × Y, S × Y and N × Y were also found to be significantly different at booting and 50% heading stages (Figure 4.a, b, c). During 2010, the highest brix value of 16.1 and 26.7% was observed in JS-2002 while the lowest 8.1 and 17.4% was in Chakwal sorghum during 2009. Similarly, the highest brix value of 16.2 and 27.6% and 18.2 and 31.8% was recorded where sulphur and nitrogen were applied @ 40 and 60 kg ha^{-1} in 2010. Interactive effect of S × N × Y was found maximum brix value of 34.3% was recorded when sulphur and nitrogen was applied @ 40 and 60 kg ha^{-1}, respectively during the year 2010 while minimum 13.5% was in control plots of both nutrients in 2009 (Figure 5).

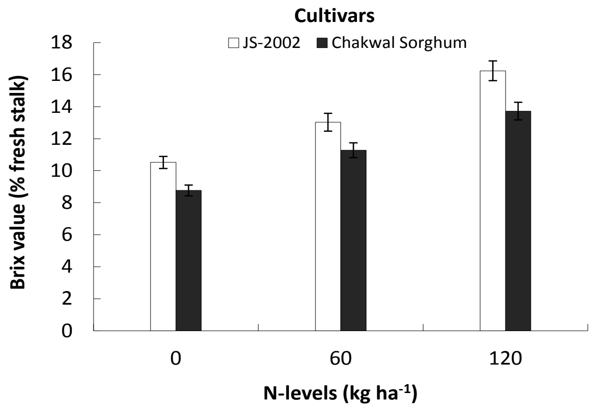

Figure 3. Effect of cultivar and nitrogen levels interaction on brix value (% fresh stalk) at booting stage. Error bars represent the standard error (n = 5).

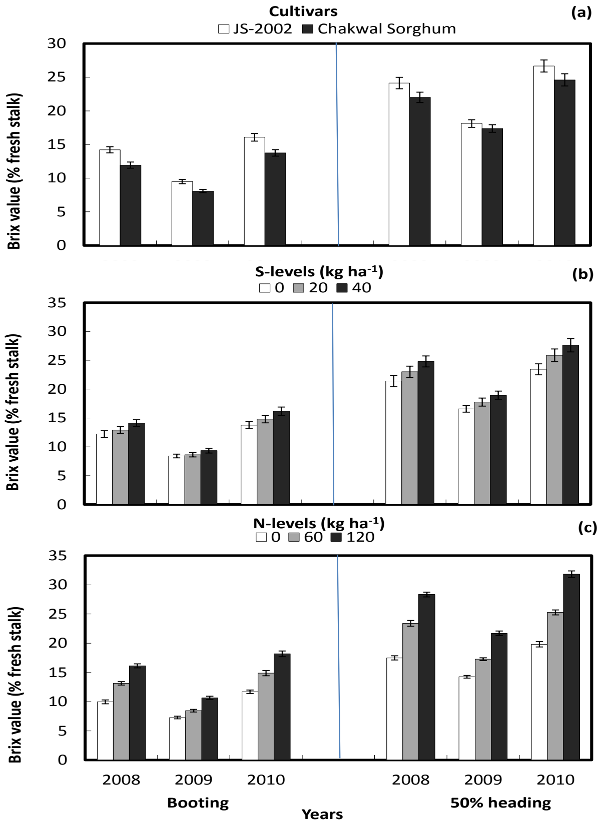

Figure 4. Effect of cultivar, sulphur and nitrogen levels interaction on brix value (% fresh stalk) at booting and 50% heading stage during 2008, 2009 and 2010. Error bars represent the standard error (n = 5).

CONCLUSIONS

The finding of three year field investigations clearly demonstrated that increased application of N resulted in increased content of HCN and brix in the forage sorghum. Therefore there is a need to explore sorghum genetic source which can result higher green forage yield with low HCN content. The application of S increased contents of brix while reduced the HCN content irrespective of sorghum cultivars. Therefore, our research work is a step towards investigation to explore balanced N and S fertilization ratio for reduced HCN content and increased sugar content in sorghum either for livestock or milling for industrial purposes.

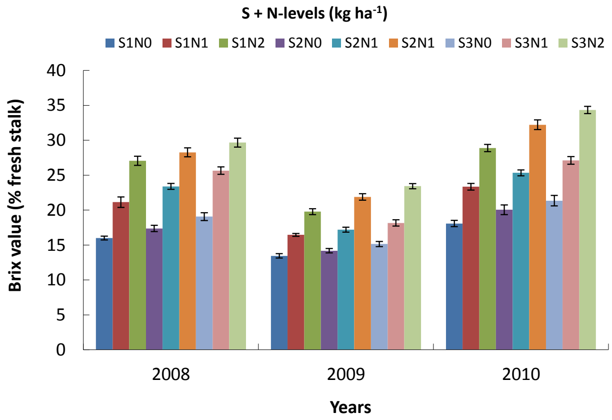

Figure 5. Effect of nitrogen and sulphur levels interaction on brix value (% fresh stalk) at 50% heading stage during three consecutive years (2008, 2009 & 2010). Error bars represent the standard error (n = 5).

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