



Full Length Article

Hydrocyanic Acid Content Variation amongst Sorghum Cultivars Grown with Varying Seed Rates and Nitrogen Levels

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ABSTRACT

Sorghum is capable of sustaining under high temperature and minimal soil moisture. Hydrocyanic acid toxin production is a serious problem associated with sorghum forage in dry areas and could be lethal to animals if ingested in higher quantities. Hydrocyanic acid (HCN) contents in forage sorghum were studied through field experiment executed at National Agricultural Research Centre, Islamabad during 2008 and 2009. Three sorghum cultivars JS-2002, Chakwal sorghum and Local sorghum were sown in a randomized complete block design in split-split plot arrangement with three seeding rates (75, 100 & 125 kg ha⁻¹) and three nitrogen levels (0, 60 & 120 kg N ha⁻¹). Increased nitrogen application progressively increased HCN production irrespective of cultivar and growth stage of the crop. Application of 120 kg N ha⁻¹ recorded about 64% higher HCN than control treatment indicating the risk of higher doses of nitrogen fertilizer to sorghum forage in dry areas. Seeding rates had variable influence on HCN production. Cultivar JS-2002 produced 23, 36 and 57% less HCN contents compared to local sorghum at pre-booting, booting and 50% heading stage, respectively. On the basis of two year's field study, it may be concluded that approved cultivar JS-2002 produced less HCN sown with higher seed rate and applied low doses of N fertilizer under dryland farming conditions of Pothwar is recommended for general cultivation. © 2012 Friends Science Publishers

Key Words: Management techniques; Forage sorghum; Nitrogen levels; Seeding rate; Hydrocyanic acid; Growth stages

Abbreviations: a.s.l.- above sea level; Cv- cultivar; DAE- days after emergence; DAS- day(s) after seeding; GDP- gross domestic product; GS- growth stages; HCN- hydrocyanic acid; N- nitrogen; P- phosphorus; S- seeding rate; Y- year.

INTRODUCTION

Livestock is an important sector for livelihood in dry land areas of Pakistan. It adds 55.1% value to agriculture and 11.5% to GDP. 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 (GOP, 2010-2011). There is need to emphasize for increased fodder production so that we could provide more support to livestock industry in the country.

Sorghum (*Sorghum bicolor* (L.) Moench) 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 is considered to be a good feed in ordinary conditions but when it's normal growth is constrained by drought (Fjell *et al.*, 1991), or imbalanced soil nutrients, hydrocyanic acid (HCN) content may develop to such an extent that the toxic level may reach lethal level when fed to animals (Singh *et al.*, 1983). Cyanide occurs in the leaves of

sorghum as cyanogenic glucoside dhurrin. Degradation of dhurrin yields equimolar amount of hydrocyanic, glucose and P-hydroxybenzaldehyde (P-HB) (Francis *et al.*, 1988). During environmental stress and when leaf tissues are cracked large amount of dhurrin may be produced rapidly. It is observed that when HCN is readily absorbed into the blood stream of grazing ruminants, it causes cellular asphyxiation and eventually leads to death of animals (Hoveland & Monson, 1980). HCN production in forage is responsible for losses in livestock and hence, a great financial loss to poor rural livestock farmers of dry areas.

No forage production system seems to be complete without selection of suitable varieties, sowing density and fertilization, which influence the plant characteristics. Farmers use these practices to improve the quality of forage, in addition to enhance the early or delayed cut of forage. Nitrogen application and sowing density directly influences the quality of the forage by changes in population dynamic. The farmers are generally not familiar with the growth stage of forage sorghum that should be feed to the livestock. They apply nitrogen fertilizer to get the higher forage yield of sorghum and feed at any growth stage without having the knowledge of HCN poisoning and its relation with these

practices. Different varieties of sorghum develop variable levels of HCN when grown under different environmental conditions. Hydrocyanic acid content is heritable and subjected to modification through selection and breeding, as well as by climate, stage of maturity, stunting of plant, type of soil and fertilizer (Khatri *et al.*, 1997). Nitrogen application is considered essential for growth and regrowth during growing season. However, higher level of nitrogen application may increase prussic acid contents of forage sorghum; ultimately poisoning animals (Aziz-Abdel & Abdel-Gwad, 2008).

Optimum plant density and nitrogen application are essential requisite to utilize the available soil and environmental resources effectively. In this study, an effort was made to manage the hydrocyanic acid through agronomic techniques in forage sorghum grown under rainfed conditions of Pakistan.

MATERIALS AND METHODS

Management of hydrocyanic acid content through agronomic techniques in forage sorghum was studied through field experiments carried out at National Agricultural Research Centre (NARC), Islamabad (33° 43' N; 73° 04' E; 518 m a.s.l) during summer, 2008 and 2009. The meteorological data during the crop growth period for both the years were collected from the Water Resources Research Institute field station, National Agricultural Research Centre, Islamabad and are presented in Table I. The soil of experimental site was a clay-loam with pH around 7.0. At both soil depths (0-15 & 15-30 cm), the organic matter and total N content was low during 2009 as compared to 2008, especially in the deeper layer. The P soil status was poor ($< 5 \text{ mg kg}^{-1}$ determined by Olsen & Sommers, 1982 method) during 2008 as compared to 2009.

Sorghum cultivars i.e., JS-2002 (V_1), Chakwal sorghum (V_2) and Local sorghum (check), were tested with three seeding rates of 75 (S_1), 100 (S_2) and 125 kg ha^{-1} (S_3) in combination with nitrogen rates of 0, 60 and 120 kg N ha^{-1} in the form of urea. Thus 27 treatment combinations were arranged in a randomized complete block design in split-split plot arrangement and replicated thrice. Sowing was done on 20th August, 2008 and 1st August, 2009 keeping similar plot area of 4.8 m^2 during both the years. The crop was sown in 30 cm apart rows with hand drill on well prepared seedbed. A basal dose of phosphorus @ 30 kg ha^{-1} (P_2O_5) in the form of triple super phosphate was applied at the time of seedbed preparation while application of nitrogen was made as per treatment in the form of urea by broadcasting in the respective plots after 10 days of sowing. All other agronomic practices and recommendations were kept as prescribed for sorghum forage crop for both the growing seasons. The total rainfall received during the crop growth period (July-August) was 538 and 278 mm during

2008 and 2009, respectively (Table I).

Fresh Leaves of sorghum were sampled at pre-booting (35 DAE), booting (45 DAE) and 50% heading (55 DAE) stage and cut into small pieces with scissors, thereafter ground up with a mortar and pestle. A round filter paper disc loaded with buffer solution of pH 6 was placed in a flat bottom plastic bottle. In the bottle 100 mg ground leaves were taken and 1 ml of distilled water was added. A yellow picrate paper was attached to a plastic stripe in such a way that the picrate paper did not touch the liquid in the bottle. The bottle was closed with a screw capped lid. Blank was used with all the same prescribed procedure but with no leaves. The two bottles were allowed to stand for 16-24 h at room temperature (20-25°C). The plastic stripe from the picrate paper was removed carefully. The picrate paper was immersed in 5 mL distilled water for about 30 min with occasional gentle shaking. The blank picrate paper was immersed in 5 mL water for about 30 min with occasional gentle shaking. Picrate papers were removed from both bottles after 30 min. The absorbance of the picrate solution was measured through spectrophotometer at 510 nm. The total cyanide content in mg kg^{-1} were calculated by the formula described by Bradbury *et al.* (1999) as follows.

$$\text{Total cyanide contents (mg kg}^{-1}\text{)} = 396 \times \text{absorbance reading.}$$

The data collected were subjected to analysis of variance using the Co. Stat 6.3 software (CoHort Software, Monterey, CA, USA) to determine significance of the tested factors (cultivar, seeding rate & nitrogen level) and their interactions with sorghum traits. The Scott - Newman-Keuls test ($P \leq 0.05$) was adopted to separate means of statistically significant sources. Pearson's correlation (r) of HCN was assessed among cultivars and nitrogen levels.

RESULTS AND DISCUSSION

Statistically significant differences for HCN content of sorghum leaves were exhibited by cultivars under varying seeding rates and nitrogen levels at all crop growth stages (Table II). The maximum HCN contents of 98.1, 68.0 and 49.1 mg kg^{-1} at pre-booting, booting and 50% heading stage, respectively were recorded by local sorghum (check). Sorghum cv. JS-2002 produced 23, 36 and 57% less HCN as compared to local sorghum and 10, 12 and 46% less HCN in comparison with Chakwal sorghum at pre-booting, booting and 50% heading stage, respectively. The difference yielded by sorghum cultivars may be result of variation in their genetic makeup (Hanuman *et al.*, 2008). Abusuwar and Hala (2010) observed higher HCN content in Abu Sabein variety, while Pandey *et al.* (2011) found it to be higher in hybrid varieties. Sarfraz *et al.* (2012) reported that variety Hegari produced higher HCN content than the other varieties. In our findings, local sorghum produced higher HCN content as compared with JS-2002 as well as Chakwal sorghum.

Table I: Weather data of experimental site during 2008 and 2009

Months	Rainfall (mm)		Mean Temperature (°C)		Relative humidity (%)	
	2008	2009	2008	2009	2008	2009
June	272.7	15.0	28.4	29.6	73.4	47.4
July	333.7	78.5	28.1	30.6	81.0	60.7
August	129.6	153.9	28.0	28.7	78.2	79.2
September	75.1	45.1	25.6	27.3	71.5	70.1
October	28.4	8.9	23.1	21.7	68.0	60.1
November	17.6	14.9	16.9	15.7	62.9	60.9
Total/Mean	857.1	316.3	25.0	25.6	72.5	63.1

Table II: Interactive effect of Cultivar, Seeding rate and Nitrogen level at different growth stages of forage sorghum during 2008 and 2009

Treatments	Hydrocyanic acid (mg kg ⁻¹)		
	Pre-booting	Booting	50% heading
Cultivar (C)			
JS-2002	75.5 c	43.7 c	21.1 c
Chakwal sorghum	84.4 b	49.9 b	27.6 b
Local sorghum	98.1 a	68.0 a	49.1 a
<i>P</i>	<0.001**	<0.001**	<0.001**
Seeding rate (S) (Kg ha⁻¹)			
75	78.2 b	46.5 b	26.3 b
100	113.2 a	74.6 a	53.1 a
125	66.6 c	38.7 c	18.4 c
<i>P</i>	<0.001**	<0.001**	<0.001**
Nitrogen level (N) (Kg ha⁻¹)			
0	67.1 c	40.5 c	21.2 c
60	88.8 b	55.5 b	33.8 b
120	102.0 a	65.6 a	42.9 a
<i>p</i>	<0.001**	<0.001**	<0.001**
Year (Y)			
2008	76.9 b	47.8 b	29.6 b
2009	95.1 a	60.0 a	35.6 a
<i>P</i>	<0.001**	<0.001**	<0.001**
C × S			
<i>P</i>	<0.001**	<0.001**	<0.001**
C × N			
<i>P</i>	<0.001**	<0.001**	<0.001**
S × N			
<i>P</i>	<0.001**	<0.001**	<0.001**
C × S × N			
<i>P</i>	<0.001**	<0.001**	<0.001**
C × Y			
<i>P</i>	0.777 ns	0.088 ns	0.061 ns
S × Y			
<i>P</i>	0.104 ns	0.687 ns	<0.003*
C × S × Y			
<i>P</i>	0.999 ns	0.331 ns	0.122 ns
N × Y			
<i>P</i>	<0.031*	0.976 ns	<0.001**
C × N × Y			
<i>P</i>	0.990 ns	0.675 ns	0.643 ns
S × N × Y			
<i>P</i>	0.975 ns	0.989 ns	0.629 ns
C × S × N × Y			
<i>P</i>	1.00 ns	0.879 ns	0.812 ns
C.V. (%)	7.4	7.9	11.0

Increase in seed rate from 75 to 100 kg ha⁻¹ recorded an increase of HCN up to 61% (Table II). The maximum HCN content of 113.2, 76.4 and 53.1 mg kg⁻¹ was recorded at pre-booting, booting and 50% heading stage, respectively

with a seed rate of 100 kg ha⁻¹. When seed rate increased from 100 to 125 kg ha⁻¹, the HCN content reduced drastically to 41, 49 and 65% at pre-booting, booting and 50% heading stage, respectively. This could be probably due to canopy structure of sorghum plants as a result of variation in seed rate, which altered the microclimate within crop. The thick canopy might have lowered the temperature under foliage and increased humidity, which resulted in lowering HCN content. Inverse relationship between temperature and hydrocyanic acid content is also supportive to this assumption (Table III & IV). However, contrary of our assumption Wheeler *et al.* (1990) could not found any response of temperature on hydrocyanic acid content. Increasing seed rate from 100 to 125 kg ha⁻¹, decreased leaf HCN content thus showed inverse relationship. These results are in agreement with the findings of Zahid (2009) who reported a strong negative correlation between the HCN content and green fodder yield. The variation of HCN content may be result of relative humidity produced with in the crop canopy, which lowered air temperature and thus, may have affected the HCN metabolism in sorghum plants. Akazawa *et al.* (1959) suggested that air temperature may have affected the activation of enzymes present in sorghum enabling them to catalyze the hydrolysis of dhudrin to yield p-hydroxy benzal-dehyde, HCN and glucose. After that HCN may have extensively metabolized to asparagine and aspartic acid (Maha-devan, 1973). Application of nitrogen showed direct relationship with HCN content of sorghum leaves (Table II). Increase of nitrogen level, progressively increased the HCN content. The maximum HCN contents of 102, 65.6 and 42.9 mg kg⁻¹ was recorded at pre booting, booting and 50% heading stage, respectively, in plots where nitrogen was applied at 120 kg ha⁻¹. The minimum of 67.1, 40.5 and 21.2 mg kg⁻¹ HCN content were recorded at pre-booting, booting and 50% heading stage in control treatment, where no nitrogen application was made. Bahrani and Ghenatghehstani (2004) reported 55% increase of HCN contents in sorghum forage when nitrogen application was increased. Similarly, Wheeler *et al.* (1980) revealed 28% increase in HCN by applying nitrogen fertilizer. Aziz-Abdel and Abdel-Gowd (2008) also reported that increase in nitrogen application resulted in enhanced HCN in sorghum. Seasonal variation may affect HCN production. Variation in rainfall pattern and temperature affected HCN content in sorghum crop as both meteorological variables are directly linked with drought. Less rainfall (48%) was received during 2009 as compared to 2008; resultantly higher temperature was recorded in the year 2009 in comparison with the earlier season. Variation of rainfall and temperature caused HCN variation in sorghum leaves at different growth stages. A reduction of 19, 20.3 and 16.9% in HCN content at pre-booting, booting and 50% heading stage was recorded during 2008 as compared to 2009.

Interactive effects of C × S exhibited significant differences at all the three growth stages (Figs. 1 a, b & c). Local sorghum showed the maximum HCN content at seed

Table III: Correlation coefficient of HCN content with temperature and rainfall of three sorghum cultivars at different growth stages

Traits	GS		Temp		RF	
	2008	2009	2008	2009	2008	2009
JS-2002						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.98**	-0.99**	0.98**	0.93**	0.99**	0.11
Chakwal sorghum						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.99**	-0.99**	0.98**	0.92**	0.99**	0.06
Local sorghum						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.99**	-0.99**	0.99**	0.90**	0.99**	0.03

GS: growth stages; Temp: temperature; RF: rainfall; HCN: hydrocyanic acid contents

**Highly significant at 1 % level of significance

Table IV: Correlation coefficient of HCN content with temperature and rainfall at different nitrogen levels at different growth stages

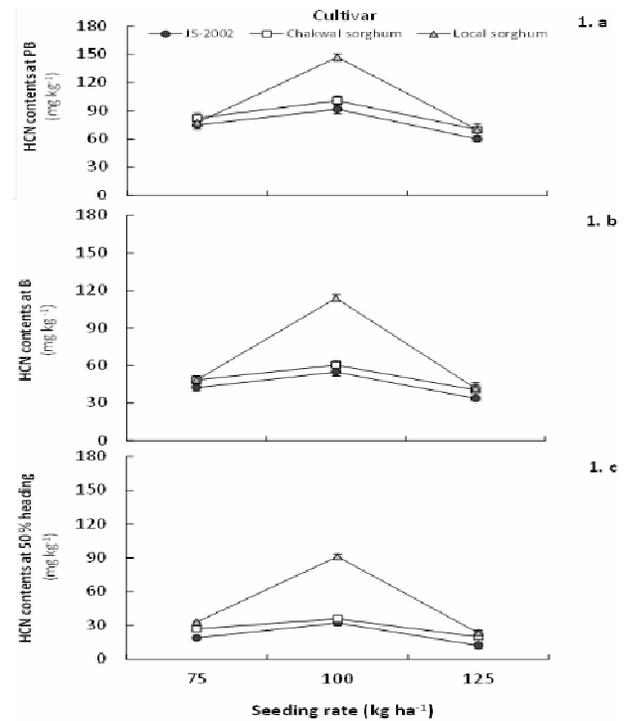
Traits	GS		Temp		RF	
	2008	2009	2008	2009	2008	2009
0 kg ha ⁻¹						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.98**	-0.99**	0.98**	0.94**	0.99**	0.13
60 kg ha ⁻¹						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.99**	-0.99**	0.98**	0.92**	0.99**	0.06
120 kg ha ⁻¹						
Temp	-0.99**	-0.95**				
RF	-0.99**	-0.17	0.99**	0.46		
HCN	-0.99**	-0.99**	0.99**	0.90**	0.99**	0.03

GS: growth stages; Temp: temperature; RF: rainfall; HCN: hydrocyanic acid content

**Highly significant at 1 % level of significance

rate of 100 kg ha⁻¹ when compared with Chakwal sorghum and cv. JS-2002. The minimum HCN content was produced by cv. JS-2002 with a seed rate of 125 kg ha⁻¹, while the Chakwal sorghum showed the intermediate results. Interactive effects of C × N showed significance observed at different growth stages for HCN content in forage sorghum (Fig. 2 a, b & c). Sorghum cv. JS-2002 without nitrogen fertilizer application produced lower HCN content compared with high doses of N- fertilizer application. These findings were also supported by work of Khair (1999), Harms and Billy (1973) who found higher HCN content with increase of nitrogen fertilization levels in all cultivars. Interactive effects of S × N revealed significant differences at all growth stages (Figs. 3 a, b & c). Maximum HCN content of 132.7, 92.5 and 67.3 mg kg⁻¹ was recorded at pre-booting, booting and 50% heading stage, respectively, with the seeding rate of 100 kg ha⁻¹ and fertilizer dose of 120 kg N ha⁻¹. Whereas, the minimum HCN content 53.2, 29.9 and 11.0 mg kg⁻¹ was recorded at three consecutive growth stages, respectively at the seeding rate of 125 kg ha⁻¹ without fertilizer application. Interactive effects of C × S × N on HCN accumulation at different growth stages of

Fig. 1: Hydrocyanic acid (HCN) contents of three forage sorghum cultivars grown in different seeding rates at pre-booting, booting and 50 % heading stage (a, b, c), respectively. Error bar represent the standard



forage sorghum are presented in Fig. 4 a, b and c. The data showed that the three factors have contrasted HCN production. The maximum HCN content of 171.8, 139.2 and 113.2 mg kg⁻¹ was recorded at pre-booting, booting and 50% heading stages respectively by local sorghum (check) when crop was sown at seed rate of 100 kg ha⁻¹ and applied nitrogen @ of 120 kg N ha⁻¹. The minimum HCN content of 54.6, 31 and 14 mg kg⁻¹ was recorded at all three growth stages for cv. JS-2002, sown with seed rate of 125 kg ha⁻¹, without fertilizer application. The only significant interaction of S × Y was observed at 50% heading stage (Fig. 5), while the interaction at other two stages was non-significant. The higher HCN contents of 57.2 mg kg⁻¹ were recorded at 50% heading stage during 2009 than 2008 at a seed rate of 100 kg ha⁻¹. Interactive effects of N × Y also showed significant differences at pre-booting and 50% heading stages (Figs. 6 a & b). The highest HCN content of 112.5 and 47.7 mg kg⁻¹ was recorded at pre-booting and 50% heading stages, respectively, during 2009 where fertilizer application was applied @ 120 kg N ha⁻¹. Conversely, the minimum HCN content were recorded during 2008 where no fertilizer was applied. In present experiments, HCN contents were higher during 2009 as compared to 2008, which may be due to less rains and higher temperature during the growth period. During 1st year of study two-fold higher rainfall received during crop season. Similarly, 10% higher temperature was recorded in

Fig. 2: Hydrocyanic acid (HCN) content at pre-booting, booting and 50% heading stage (a, b, c) of three forage sorghum cultivars grown with different nitrogen levels. Error bar represent the standard

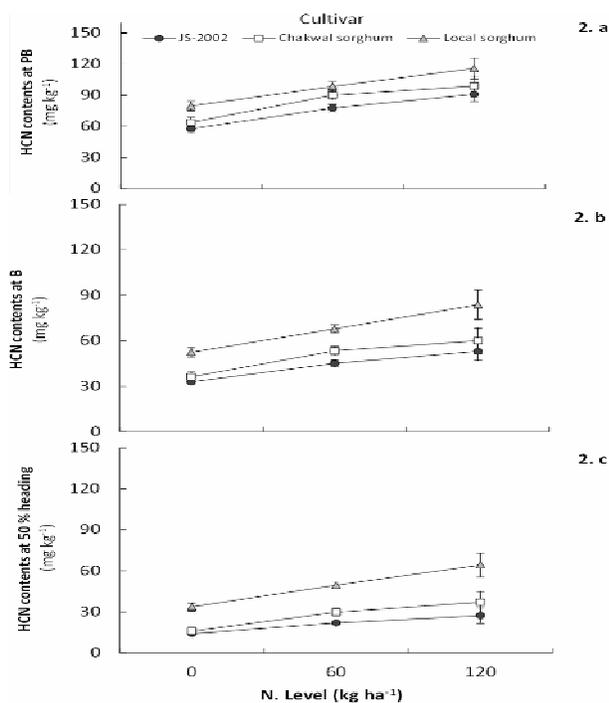


Fig. 3: Effect of seeding rate and N- level interactions on hydrocyanic acid (HCN) content of forage sorghum at pre-booting, booting and 50% heading stage (a, b, c). Error bar represent the standard

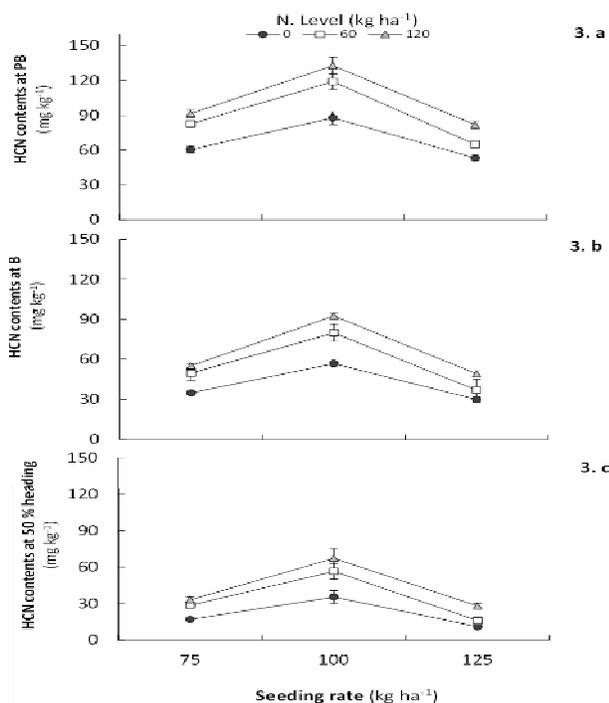


Fig. 4: Effect of cultivar, seeding rate and nitrogen interactions on hydrocyanic acid (HCN) content of forage sorghum at pre-booting, booting and 50% heading stage (a, b, c). Error bar represent the standard

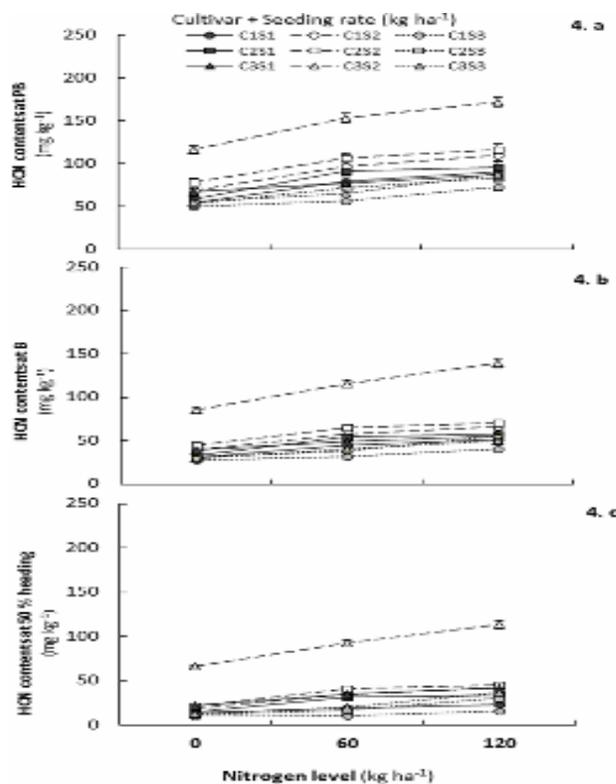
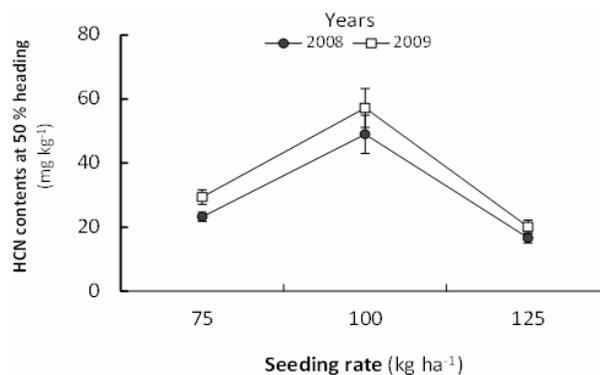
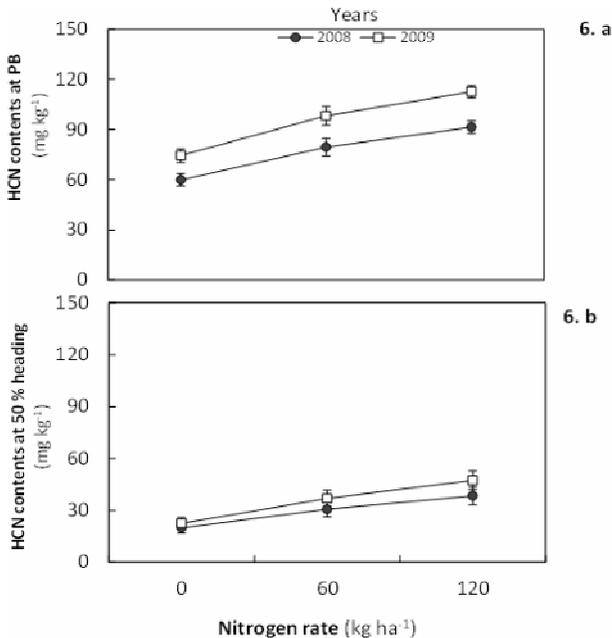


Fig. 5: Effect of seeding rate and seasonality on hydrocyanic acid (HCN) content of forage sorghum at 50% heading stage. Error bar represent the standard



the later year during crop growth period (Table I). These results are in line with the findings of Kumar and Devender (2010) who concluded that seasonal differences in HCN were significant and plants at ages 45-60 DAS were with safe levels of HCN content in sorghum forage. The safe level of HCN is below 200 mg kg⁻¹ on wet weight basis and HCN level more than this in sorghum forage is dangerous

Fig. 6: Effect of different nitrogen level and seasonality on hydrocyanic acid (HCN) content of forage sorghum at pre-booting and 50% heading stage (a, b). Error bar represent the standard



for livestock (Fjell *et al.*, 1991).

Perusal of Table III indicated significance (negative) association between growth stages (GS) and temperature ($r: -0.99$), rainfall ($r: -0.99$) and HCN ($r: -0.98$) during 2008, while GS showed significant (negative) correlation with temperature ($r: -0.95$) and HCN ($r: -0.99$) for JS-2002, chakwal and local sorghum cultivars during 2009. Similarly temperature had significant and positive correlation with rainfall during 2008, whereas with HCN association was significant and positive during 2008 and 2009 for the same sorghum cultivars. Rainfall showed significant and positive relationship with HCN ($r: 0.99$) during 2008 for different sorghum cultivars. Table IV showed that growth stages (GS) had significant and negative association with temperature ($r: -0.99$), rainfall ($r: -0.99$) and HCN ($r: -0.99$) during 2008 while the relationship of GS with temperature ($r: -0.95$) and HCN (-0.99) was found significant and negative for different levels of nitrogen under study during 2009. Temperature depicted significant and positive association with rainfall ($r: 0.99$) and HCN ($r: 0.98$) during 2008, while significant and positive with HCN ($r: 0.94$) during 2009. Rainfall displayed significant and positive relationship with HCN ($r: 0.99$) during 2008 for different levels of nitrogen under evaluation. Present results clearly indicated that HCN poisoning can be managed by avoiding the seasonal severities and management practices for provision of safe sorghum forage to livestock in dryland farming. The results showed that advancement in the growth stages decreased HCN contents in sorghum. Similarly increase in rainfall decreased HCN contents in sorghum plants.

CONCLUSION

From the results of two years field experiments, it may be concluded that management strategies in combination with genetic variability could produce safe sorghum forage free HCN toxin. Sorghum forage cultivar JS-2002 with seed rate of 125 kg ha^{-1} without N application produced safe forage for livestock at 50% heading stage under dryland farming conditions of Pothwar. However, variation of rainfall and temperature during crop growth period would affect HCN concentration. HCN accumulation increased with the rise of temperature while reverse was true for seasonal precipitation.

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