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Determination of HCN Content in Some Silage Sorghum Genotypes

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Abstract
The aim of the study was to determinate that hydrocyanic acid (HCN) content. The experiment was carried out at Field Crop Department of Bati Akdeniz Agricultural Research Institute, in Antalya, in Turkey in 2017. The experiment was consisted of forty two genotypes of silage sorghum. The study was laid out in a randomized plot design with three replications. After harvesting, the seed samples were analyzed to determine the amount of HCN. The collected data were analyzed and the means were done multiple range tests at 1% level of probability by Duncan's. Among the genotypes, showed significantly differences with regard to HCN content. The HCN ratio in sorghum genotypes were varied between 8.2 and 54.4. The highest HCN content were detected in 3, 17, 111, 157, 168 and 214 genotypes. As a result of the trial, it can be said that the HCN content may be used breeding criteria.

Keywords: Sorghum genotypes; Breeding criteria; HCN content

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is grown essentially for animal feed. It is important forage crop in many regions of the world in rainfed conditions [1]. The sorghum grain is used both for human nutrition and animal feed in Africa and Asia. More than 35% of sorghum is grown directly for human consumption. It is estimated that more than 300 million people from developing countries essentially rely on sorghum as source of energy [2]. Sorghum is genetically diverse. At the International Crop Research Institute are deposited holds about 36.000 germplasm accessions of sorghum. The varieties are distinguished on the basis of morphological traits, differences in isoenzyme patterns and DNA polymorphism ([3]; [4]; [5]). Sorghum is known for its nutritional quality. Sorghum is a good source of the B vitamins and the liposoluble vitamins A, D, E and K also a good source of more than 20 minerals [6]. The future promise of sorghum in the developed world is for wheat substitution for people allergic to gluten [7]. Furthermore sorghum's starch is successfully applied for the production of bio-ethanol [8]. However, though roughage is the cheapest form of feed for animals but the present fodder production does not meet the requirement in terms of quantity and quality [9]. The major factors limiting the sorghum fodder production are related to specific growth stages, insufficient fertilizer application and high contents of HCN [10]. The contents of the HCN in sorghum vary depending on plant growth stage, genotype (variety) and environmental conditions [11]. There is a rapid increase in the cyanide potential of sorghum during germination and early seedling formation; thereafter it declines with plant growth stages [12]. Furthermore Pirincci and Tanyıldızı, 1994 [13] were investigated HCN levels in the 30 grains and 13 feeds obtained from different regions and they found HCN levels in the sarnples ranged from o. 14 to 48.96 ppm.

HCN levels in sorghum are important, whether in human nutrition or animal consumption. Hence, it is essential to develop varieties with high forage/seed yields and low hydrocyanic acid (HCN) content in sorghum. For this purpose the study was conducted to determine whether differences for HCN content in sorghum genotypes.

MATERIALS AND METHODS

Plant Material and Growth Conditions;

The study was conducted in the field crops department of Batı Akdeniz Agricultural Research Institute in 2017. The experimental field was under Mediterranean climate conditions. The climate data of experimental area, maximum, minimum and average temperatures and rainfall for 2016, 2017 and longtime were shown Table 1.

Table 1. Climate Data (Long Period-2016 and 2017)

Month	Precipitation			Temperature(^{0}C)			Relative humidity (%)		
	Long Period	2016	2017	Long Period	2016	2017	Long Period	2016	2017
January	245.7	236.3	114	10.2	9.8	8.8	67.2	66.3	64.6
February	133.2	156.2	8.8	11.1	10.4	11.1	67.1	67.4	63.6
March	48.2	98.8	95	13.7	12.7	13.3	66.4	69.5	69.7
April	55.8	52.5	34	16.4	16.1	16.9	67.1	64.3	66.4
May	49.8	31.5	59.6	21	20.5	20.7	66.6	68.9	70.7
June	4.2	9.4	0.2	25.9	25.4	26.2	61.2	61.2	63.9
July	3	2.5	0	28.9	28.4	29.9	60.3	62.3	54
August	1.8	2.7	0	28.8	28.2	28.9	62.9	70	53.8
September	27	14.5	0.4	25.1	24.7	25.3	61.3	60.6	70.3
October	134.4	72	44.6	20.3	20	20.2	62.7	62.4	59.8
November	77.8	131.4	80.7	15.4	14.9	16.5	66.5	65.9	64.6
December	182.5	261.1	75.4	11.6	11.3	12.8	66.2	56.4	61.3
Total	963.4	1069	512.7	-	-	-	-	-	-
Mean	-	-	-	19.03	18.53	19.22	64.62	64.6	63.5

During the experiment in 2016 and 2017, total annual precipitation was measured as 1069.0 and 512.7 mm respectively. Long period average precipitation of the experimental area is 963.4 mm. Irrigation was done when it is necessary (in 04.06.2017, in 01.07.2017 and in 01.08.2017) because of most of rainfall took place in winter months. After that, irrigation was not done until the end of the experiment. In terms of relative humidity and average temperature of 2016 and 2017 were similar to long period.

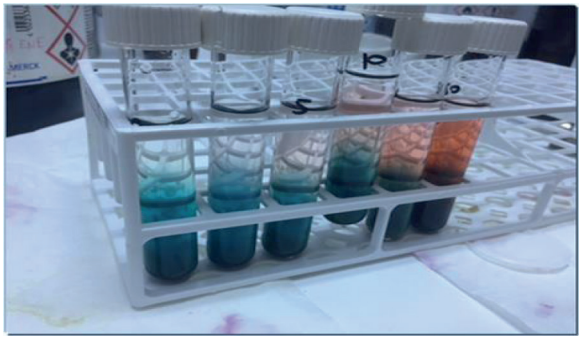
The research was conducted in the plot that have silty with clayey, saltless, very high limey, strong alkaline and low organic matter content soil. Sorghum line's seeds were sown in 3 m long plots with 8 rows. Row spacing and intrarow spacing were 45 cm and 10 cm respectively. Seeds were sown 2 kg per decare. In this experiment, 4 kg da-1 21 % ammonium sulfate and 8 kg da-1 42 % triple super phosphate were applied with sowing and any fertilizer treatments were not done throughout the growth season. The study was established in a randomized plot design with three replications and planted in 11.05.2017. Weed control was done manually in growing season. All other agronomic practices were done during the growing seasons. Plants seeds were harvested at 09.08.2017

Extraction Procedure of Sample;

Seeds were ground to powder and 0.5 g sample was taken with 0.1 mg sensitivity, then 9.5 ml methanol (80%) was added to them and samples were extracted on orbital shaker for 24 hours under room conditions. The extract mixture was centrifuged at + 4 °C for 10 minutes and was filtered. The seed residues were re-extracted in the same way at three times. After filtration the seed residues, the three extract were combined. This method was modified by the methanol extract method of Cai, et al., 2004 [15].

Analysis Procedure of HCN;

HCN levels in the seed samples were determined using the colorimetric method that is suggested by Lambert et al. 1975 [14] and adapted by Pirincci and Tanyıldızı, 1994 [13]. According to this method; calibration curve was prepared using by 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, 1, 2, 3, 4, 5, 6, 8, 10 and 20 ppm potassium cyanide solutions (Sigma-Aldrich Cat. No:31252-100 g) (Figure 1). 1 ml water samples, 1 ml N-chlorosuccinimide-succinimide solution (10 g succinimide Sigma- Aldrich Cat. No: 59381-500 g was dissolved in 200-300 ml distilled water, afterwards, 1 g N-chlorosuccinimide Sigma- Aldrich Cat. No: 109681-100 g was added and the solution was completed to the 1 lt with distilled water) and 1 ml barbituric acid-pyridine solution (3 g barbituric acid Sigma-Aldrich Cat. No:185698-25 g was dissolved in 10 ml distilled water, afterwards, 15 ml pyridine Sigma-Aldrich Cat. No:360570-100 ml was added and the solution was completed to the 50 ml with distilled water) were mixed and dropped to the 25 ml flasks, afterwards, the solution was completed to the 25 ml with distilled water.



The solution was held on 15 minutes in the darkness for the color formation. After that, HCN levels of the samples were determined as ppm by observation of absorbance in 575 nm in the spectrophotometer (Shimadzu, UV 1600).

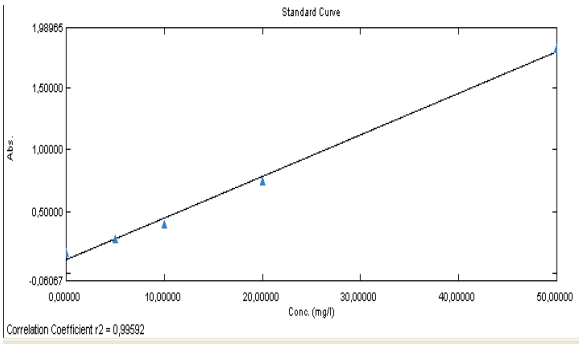


Figure 1. Standart curve

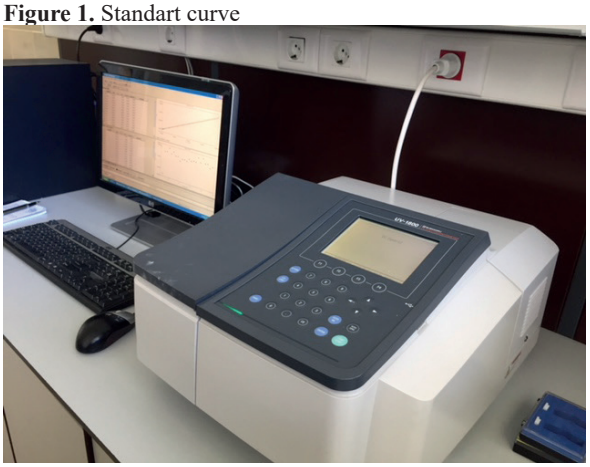


Figure 1. Spectrophotometer

Statistical Analysis;

All obtained data in the experiment were analyzed using the SAS, 1998 [16] program. Analysis of variance was performed and means were separated by Duncan Multiple Range Test.

RESULTS AND DISCUSSION

According to the results of analysis of variance there was found highly significant at the 0.01 level for HCN content (Table 2). Duncan test for means of HCN levels was stated at Table 3.

Table 2. Analyzis of variance of HCN content in sorghum lines

Source of Variation	DF	Sum of Squares	Prob
Replication	2	10.189	0.3767
Line	41	22707.854	<.0001**
Error	82	422.771	
Total	125	23140.814	
CV (%)	6.74		

It was observed that HCN level low in 98, 106, 183, 10, 161, 94, 162, and 29 lines (15.20, 15.20, 15.20, 14.60, 11.80, 11.00, 10.40 and 8.20 ppm) and high in 214, 3 17 157 111 168 and 169 lines (54.40, 53.80, 53.60, 52.40, 52.00, 50.80 and 48.20 ppm)

The HCN content of dry sorghum seeds was generally low and varied from 8.20 ppm to 54.40 ppm. Panasiuk and Bills 1984 [17] observed that dry sorghum seeds contained from 1 or 2 to 29 ,ug of cyanide per gram. The difference yielded by sorghum cultivars may be result of variation in their genetic makeup [18]. Pirincci and Tanyıldızı, 1994 [13] investigated HCN levels in the 30 grains and 13 feeds obtained from different regions and they found HCN levels in the samples ranged from 0.14 to 48.96 ppm. It can be said that similar varietal as well as locational differences in the HCN content of sorghum seeds.

Table 3. HCN contents of lines

Line No	HCN Content (ppm)	Line No	HCN Content (ppm)	Line No	HCN Content (ppm)	Line No	HCN Content (ppm)
214	54.40 a	**18**	43.00 df	**6**	33.20 gj	**84**	15.80 mn
3	53.80 a	**79**	42.60 df	**45**	33.20 gj	**98**	15.20 mo
17	53.60 a	**44**	41.60 df	**46**	32.80 gj	**106**	15.20 mo
157	52.40 ab	**153**	40.20 eg	**154**	32.40hj	**183**	15.20 mo
111	52.00 ab	**72**	38.80 eh	**37**	31.00 ık	**10**	14.60 mo
168	50.80 ac	**1**	38.20 fı	**136**	30.80 ık	**161**	11.80 no
169	48.20 ad	**80**	38.20 fı	**15**	29.40 jk	**94**	11.00 no
164	46.00 be	**2**	37.40 fı	**20**	26.80 jl	**162**	10.40 no
100	44.40 cf	**19**	37.40 fı	**16**	24.80 kl	**29**	8.20 o
74	44.00 cf	**38**	37.40 fı	**110**	24.40 kl		
163	43.60 cf	**49**	37.40 fı	**36**	21.80 lm		

CONCLUSION

In conclusion, forty two sorghum lines that have different origins were used, and it was investigated that whether the HCN content could be used as a selection criterion in this experiment. In this study, it was monitored that large differences HCN content were observed in all lines. Sorghum lines used in the experiment have a different origin. It can be said that besides not only genetic differences, different origin has an effect on HCN content of sorghum seeds also. As a result; it can be said that HCN content of sorghum could be used as a selection criteria in sorghum breeding program.

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