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Preliminary Report on Inheritance of Differential Ability of Inbred Lines of Sudan Grass to Produce HCN

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LOSSES of cattle pasturing on sudan grass [*Sorghum vulgare* var. *sudanense* (Piper) Hitch.] have been reported at various times. The lethal properties of these pastures are assumed by some workers to be due to plants with high concentrations of hydrocyanic acid (HCN). The amount of HCN may be influenced by: (1) A mechanical mixture of sudan grass with the forage sorghums that are high in HCN, (2) the presence of sudan x sorghum hybrids, and (3) the ability of sudan grass to produce extremely high concentrations of HCN. A rather complete literature review of cyanogenesis in plants has been compiled by Leemann.[1]

The present study was designed to determine whether or not selfed lines of sudan grass and their progeny differed significantly in HCN content. Much of the work reported on HCN content of sudan grass has been carried out on commercial strains. Recently, Rodgers, et al.[2] found no relationship between seed characteristics and HCN content of sudan grass plants grown from the same lot of seed. Since sudan grass is an often cross-pollinated crop, there is considerable variability in the complex making up a commercial strain. Self-fertilized, i. e., selfed lines, of sudan grass were used in this study in order to overcome this variability.

Experimental Procedure

Material

Several plants in a field of apparently pure sudan grass were selfed in the summer of 1933. In succeeding years selfing was continued in the original lines. From 13 original selfed plants in 1933, 38 S_3 (selfed three generations) lines were grown in 1936. From these 38 S_3 lines, 112 selfed lines were grown in 1937.

Methods

The 38 S_3 lines were planted in single-head rows in 1936. In 1937 three randomized blocks, each of which included one S_4

[1] Leemann, A. C., "Hydrocyanic Acid in Grasses," *Ond. Jour. of Vet. Sci. and Anim. Ind.*, 5:97-136. 1935.

[2] Rogers, C. F., Larson, A. H., and Spracher, M. L., "Variations in Hydrocyanic Acid Content of Sudan Grass from a Single Lot of Seed," *Jour. Amer. Soc Agron.*, 29:865-876. 1937.

from each of the 38 S_3 lines, were planted. Two of the S_4 lines failed to germinate. In both years the rows were 3 feet apart and 30 feet long.

Tillers were harvested just above the ground from eight to ten plants per inbred line and bulked into one composite sample. The samples were taken at the late boot stage in 1936 and at the early heading stage in 1937.

Each composite sample was placed in a 1-liter, open-mouthed bottle sealed with a rubber stopper, taken into the laboratory, ground in an ordinary hand-power rotary food grinder, and duplicate 50-gram samples weighed into a 500 cc Erlenmeyer flask, with enough water added to cover the sample. The flasks were sealed with rubber stoppers. The next morning distillations were made in the Kjeldahl equipment.

The 1936 samples were analyzed by the sulfocyanate red colorimetric method[3], but the 1937 samples were analyzed by the modified alkaline method[4] after a comparison of various methods was made in the laboratory[5].

All results reported in p. p. m. HCN of green plant material.

EXPERIMENTAL RESULTS

The inbred lines of sudan grass differed in many respects. Some of the lines were quite uniform for various characters, while others were still segregating. The lines differed in such characteristics as glossy and non-glossy leaves, purple-tipped and non-purple tipped seedling leaves, and broomcorn and other panicle types. Glume color ranged from a light straw color through the reds, brown, and near blacks. Some lines had seeds that were long and narrow, while others had short plump seeds, but none had seeds so plump and large as those of Black Amber sorghum. The narrow seed of some of the lines dehisced similar to Johnson grass. There was a difference of about 2 weeks in date of maturity between the selfed lines. Leaf width varied from 0.5 to 1.25 of an inch, height ranged from 4 to 7 feet, culm diameter fluctuated from 0.16 to 0.29 of an inch, forage yield (air-dry weight) had a low of 1.5 tons per acre and a high of 5.75 tons per acre, and percentage of lodging ranged from 4 to 29. Aphid infestation varied from 5 to 44 percent, while grasshopper damage fluctuated from 3 to 66 percent. All the

[3] Johnson, Maxwell O., "On the Determination of Small Quantities of Hydrocyanic Acid," *J. A. C. S.*, 38:1230-1235. 1916.

[4] "Alkaline Titration Method," *J. A. O. A. C.*, 19:94. 1936.

[5] Coleman, O. H., and Gardner, R., *Comparison of Methods of Determining Small Quantities of HCN* (in press).

Variation in leaf width and plant height between some of the S_3 lines of sudan grass; the line second from the left is much shorter-stemmed and narrower-leafed than the other lines.

S_4 lines from one original selection were badly damaged by grasshoppers. Differences in HCN content also were observed.

In order to determine whether or not different S_1 lines of sudan grass transmit to their respective progeny differential abilities to produce HCN, the S_3 and S_4 progeny were grouped according to their origin as S_1 lines. The mean value of HCN p. p. m. for the progeny of each S_1 line is included in table 1.

TABLE 1.—*HCN (p. p. m.) in inbred lines of sudan grass, grouped according to their origin as S_1 lines, Fort Collins, Colo. (1936-37).*

S_1 line number	Number lines	S_4 1937 Mean HCN (p. p. m.)	S_3 1936 Mean HCN (p. p. m.)
137	2	232.9	47.4
138	4	80.1	20.8
147	2	135.7	34.6
150	3	102.0	24.5
152	3	201.1	31.0
153	7	140.3	45.8
185	2	208.2	15.6
214	3	133.1	24.9
228	4	132.2	23.7
322	2	155.8	24.1
386	2	136.4	19.3
387	2	164.9	40.7
388	2	185.1	67.7
High		232.9	67.7
Mean		146.8	32.6
Low		80.1	15.6

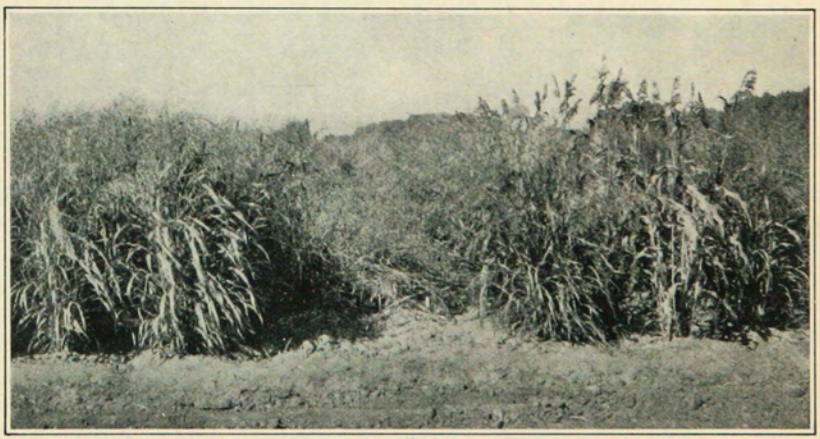
Column 1 gives the number of each of the 13 original S_1 lines. While the HCN (p. p. m.) content was lower in 1936 than 1937, the progeny of lines 138, 150, 214, 228, and 386 were low in both years, while the progeny of lines 137, 387, and 388 were high. Of the five remaining lines only one, 185, was badly reversed, being low in 1936 and high in 1937. Line 153 was high in 1936 and low in 1937. The other lines were intermediate in both years.

The statistical interpretation of these data is given in table 2. The F′ values[6] indicate that differences in HCN between years, between lines, and between interactions of lines x years

TABLE 2.—*Variance*[7] *distribution of HCN p. p. m. in inbred lines of sudan grass, grouped according to their origin as* S_1 *lines, Fort Collins, Colo. (1936-37).*

Variance due to	D. F.	Sum squares	Mean square	S. E.	Observed value F′	F″
Years	1	247,711.22	247,711.22		582.74**	
Lines	12	41,504.35	3,458.70		8.14**	1.54
Lines x years	12	26,047.67	2,170.64		5.11**	
Error	50	21,253.78	425.08	20.62		
Total	75	336,517.02				

F″ is the ratio of variance of lines to lines x years.
** > 1% point.



A line badly lodged in comparison with the stiffer lines; the row on the extreme right is an inbred line of Black Amber sorghum.

[6] Snedecor, G. W., *Calculation and Interpretation of Analysis of Variance and Covariance* (Collegiate Press Inc., Ames, Ia.) 1934.

[7] Fisher, R. A., *Statistical Methods for Research Workers* (Oliver and Boyd, Edinburgh, London, etc.) 3d ed., 1930.

cannot be attributed to chance fluctuations. Since the F'' value comparing variance due to lines with the interaction variance lines x years is low, then for this test, it appears that even though the progeny of some inbred lines were uniformly high or low in HCN production, the progeny of other lines were not uniform.

Table 3 presents the detailed data obtained from the three selfed lines selected from each of the 38 S_3 lines grown in 1936.

TABLE 3.—*HCN (p. p. m.) content in the 38 S_3 inbred lines of sudan grass and their selfed progeny, Fort Collins, Colo. (1936-37).*

S_1 line number	S_2 line number	S_4 lines HCN p. p. m.	S_4 lines HCN p. p. m.	S_4 lines HCN p. p. m.	(Mean) HCN p. p. m. 1937	S_3 (Mean) HCN p. p. m. 1936
137	A	332.4	227.3	144.5	234.7	42.7
	B	253.9	227.3	212.1	231.1	52.1
138	A	79.8	107.8	54.2	80.6	24.9
	B	115.3	81.2	52.6	83.0	25.6
	3	96.8	80.5	73.4	83.6	11.7
	5	110.6	72.8	36.2	73.2	21.1
147	A	150.1	102.6	86.6	113.1	35.8
	B	154.1	227.1	93.8	158.3	33.3
150	A	91.4	78.3	66.2	78.6	29.0
	B	132.6	—	94.4	113.5	14.6
	9	140.4	94.0	107.1	113.8	30.0
152	A	176.9	196.3	129.0	167.4	56.5
	B	190.8	227.8	141.1	186.6	15.9
	3	272.2	253.5	222.2	249.3	20.7
153	A	194.5	98.1	231.4	174.7	36.6
	B	111.3	81.0	64.0	85.4	33.3
	1	191.0	153.5	119.4	154.6	33.3
	2	142.5	215.3	92.9	150.2	66.7
	8-1	105.6	116.9	137.7	120.1	48.3
	8-2	156.6	156.7	152.3	155.2	47.0
	8-3	160.3	160.0	106.1	142.1	55.6
185	A	238.6	—	112.7	175.7	14.4
	B	306.7	220.0	195.2	240.6	16.7
214	A	156.3	165.4	184.1	168.6	27.3
	B	114.4	102.1	72.9	96.5	25.3
	C	149.1	156.8	96.5	134.1	22.2
228	1	197.6	145.2	89.8	144.2	25.1
	2	181.6	145.4	75.8	134.3	18.5
	3	120.4	109.8	97.0	109.1	25.1
	4	175.2	141.2	107.3	141.2	26.0
322	A	225.5	136.2	108.2	156.6	20.3
	B	204.8	114.3	145.9	155.0	27.8
386	A	140.8	191.3	63.9	132.0	13.7
	B	172.8	96.7	152.6	140.7	24.9
387	A	191.3	136.7	159.3	162.4	40.7
	B	201.6	174.2	126.5	167.4	40.7
388	A	197.9	154.6	95.6	149.4	61.3
	B	274.2	201.1	187.1	220.8	74.1
Mean					146.8	32.6
2 x S. E. of Diff.					54.0	—

The progeny test indicates that some uniformity in HCN content is being obtained and that some lines were persistently high and others persistently low in HCN content. Lines 137-A, 137-B, 387-A, 387-B, and 388-B were all uniformly high in 1937 and were also high in 1936. On the other hand, lines 138-A, 138-B, 138-3, 138-5, 150-A, 150-B, 150-9, 214-B, and 228-3 were all uniformly low in both years. Some of the other lines varied, being high one year and low the other year, or vice versa. Lines 147-A, 153-1, 228-4, and 386-B were intermediate in both years.

The S_4 lines were grouped according to their S_3 parentage in order to determine whether or not these S_3 lines of sudan grass had differential abilities to produce HCN. The analysis of variance is presented in table 4. The F value of 3.47 indicates that these S_3 lines of sudan grass have differential abilities to produce HCN. Soil heterogeneity may influence the production of HCN in sudan grass, since the variance due to replications was statistically greater than that between lines or the interaction lines x replications.

TABLE 4.—*Variance distribution of HCN p. p. m. in inbred lines of sudan grass, Fort Collins, Colo., 1937.*

Variance due to	D. F.	Sum squares	Mean squares	S. E.	F	F′	F″
Between lines	37	475,405.23	12,848.79		3.47**	5.88**	
Within lines	74	273,777.80	3,699.70				
Replications	2	116,496.92	58,248.46			26.66**	4.53*
Lines x replications	72	157,280.88	2,184.46	46.74			
Plots	111						

F is the ratio of variance between lines to within lines.
F′ are the ratios of variances between lines, and replications to lines x replications.
F″ is the ratio of variances of replications to between lines.
** > 1% point.
* > 5% point.

The results of a classification of the inbred lines of sudan grass according to phenotypes are given in table 5. It is apparent that, for the lines studied, the HCN was higher in the normal or non-glossy plants than in the glossy, since the former had an average of 45.7 and 169.8 p. p. m. in HCN in 1936 and 1937, respectively, and since the latter had an average of 27.3 p. p. m. and 132.0 p. p. m., respectively, for 1936 and 1937. Both years the difference was greater than twice the standard error of the difference. Those lines with purple-tipped leaves at the seedling stage were statistically higher in HCN in 1936 than

those without the purple tipping. In 1937 the former were higher again, but the difference could be attributed to chance.

TABLE 5.—*HCN (p. p. m.) in inbred lines of sudan grass, grouped according to phenotypes, Fort Collins, Colo. (1936-37).*

Phenotype	1936 HCN p. p. m.	1936 Number lines	1937 HCN p. p. m.	1937 Number lines
All lines	32.6	38	146.8	112
Glossy	27.3	27	132.0	68
Non-glossy	45.7	11	169.8	44
2 x S. E. Diff.[6]	9.6		21.4	
Purple-tipped leaves	40.5	13	153.5	49
Non-purple tipped leaves	28.5	25	141.7	63
2 x S. E. Diff.[6]	10.1		22.1	

CONCLUSIONS

1. The progeny (S_3 and S_4 lines) test indicates that the differential ability to produce HCN may be inherited in inbred lines of sudan grass.

2. Soil and seasonal differences seem to influence the production of HCN in inbred lines of sudan grass.

3. In the lines studied, high HCN production appears to be more closely associated with non-glossy leaves than with glossy leaves. Similarly, high HCN content seems to be associated with purple-tipped seedling leaves, but to a lesser degree.