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Inheritance of Seedling Hydrocyanic Acid Potential and Seed Weight in Sorghum-Sudangrass Crosses¹

J. F. S. Lamb, F. A. Haskins, H. J. Gorz, and K. P. Vogel²

ABSTRACT

The hydrocyanic acid potential (HCN-p) of sorghum [*Sorghum bicolor* (L.) Moench] plants is recognized as a heritable trait, but previous studies on the mode of inheritance of HCN-p have produced inconsistent results. The objective of this study was to investigate the inheritance patterns of seedling HCN-p and also of seed weight in reciprocal crosses of sorghum and sudangrass [formerly *S. sudanense* (Piper) Stapf]. Both traits were found to be inherited quantitatively. Generation means analysis indicated that additive genetic effects were most important for both seed weight (53% of variation) and seedling HCN-p (74% of variation). A maternal effect was found for both traits in the F_1 and backcross generations. No evidence of this reciprocal effect was found in the F_2 , suggesting that cytoplasmic inheritance was not involved. A highly positive correlation between seed weight and HCN-p was found across all entries, across both parents, and in the F_1 and backcross generations. However, correlation coefficients between seed weight and seedling HCN-p for individual entries, the pooled F_2 's, or within types of seed parents in the F_1 or backcross generations were generally nonsignificant. It was concluded that seed weight per se does not have a large effect on seedling HCN-p.

Additional index words: Dhurrin, Prussic acid, *Sorghum bicolor* (L.) Moench, *S. sudanense* (Piper) Stapf.

PREVIOUS studies of the inheritance of the cyanogenic glucoside, dhurrin [*p*-hydroxy-(S)-mandelonitrile- β -D-glucoside], in sorghum [*Sorghum bicolor* (L.) Moench] have not yielded consistent results. Nass (17) in 1972 reviewed published reports on the inheritance of cyanogenesis in sorghum, various *Lotus* species, and white clover (*Trifolium repens* L.), and concluded that the situation in sorghum was more complex than that in other species. The studies agreed that the dhurrin content or hydrocyanic acid potential (HCN-p) of sorghum leaves was a heritable trait, but disagreed on the presence or absence of dominance, dominance of high or low HCN-p, and the number of genes involved.

Krauss (14), in a study not included in Nass's review, concluded that HCN-p was conditioned by four genes with additive effects without dominance. Other studies conducted since Nass's review reported different conclusions on the importance of additive and nonadditive effects (2, 4, 9). Recently, Gorz et al. (10) found that seedlings of sorghum lines KS8 and N32 were high in HCN-p, but the HCN-p of flag leaves from field-grown plants of KS8 was only about 1/10 as high as that of N32 flag leaves. A single gene pair was found to be primarily responsible for the large difference in mature leaves, and neither low nor high HCN-p was completely dominant. The lack of agree-

ment among these studies was no doubt due in part to differences in lines or cultivars used, conditions of growth, types of tissue assayed, and the analytical procedures used.

The primary objective of the present study was to investigate the inheritance of seedling HCN-p in crosses of grain sorghum and sudangrass [formerly *S. sudanense* (Piper) Stapf] lines. The sudangrass lines had been selected for low HCN-p; crosses of these lines to the high-HCN-p sorghum lines provided a wider range in HCN-p than was possible in most of the previous studies of HCN-p inheritance. Also, this study utilized the spectrophotometric assay described by Gorz et al. (8). This assay is independent of the activity of catabolic enzymes in the plant.

MATERIALS AND METHODS

Four inbred parental lines were used in this study. Two were lines from sudangrass populations, and two were grain sorghum cultivars. The two sudangrass lines, 81-1901-7 and 81-1904-74, hereafter referred to as 1901 and 1904, respectively, were selected primarily for low HCN-p. Both lines carried the *ms₃* gene for genetic male sterility (gms); this recessive gene caused 25 to 30% of the plants in each line to be sterile. Both sudangrass lines also restored fertility when crossed with cytoplasmic-male-sterile (cms) sorghum lines having A1 cytoplasm. The population from which line 1901 was selected has since been released and registered as NP25 low-dhurrin sudangrass germplasm (12). Line 1904 was selected from a population with most of its background from 'Piper'. Both 1901 and 1904 are very low in seedling HCN-p compared to commercially available sudangrasses, and they are considered to be homozygous for low HCN-p. The two grain sorghums used were 'Redlan' and 'Combine Kafir 60' (CK60). A-lines (A1 cytoplasm) and B-lines of these cultivars have been maintained for at least 10 yr by making paired crosses annually to produce A-line seed and also selfing the B-line plants used in the A-line crosses. The lines are considered to be homozygous for high seedling HCN-p.

All possible crosses, including reciprocals, were made among the four parental lines. Crosses were made using gms plants from the sudangrass lines and cms plants from the sorghum cultivars as females. Use of these male-sterile plants allowed for production of a large number of F_1 seeds with minimal risk of contamination during crossing. The F_1 plants were selfed to produce F_2 seed, and were also used as pollen sources in backcrosses to gms sudangrass and cms sorghum parental plants. To get backcross seed from the sorghum $\times$ sorghum F_1 's, the cms hybrids were used as females, and pollen was taken from the parental sorghum B-lines. All crosses were made at the University of Nebraska Agronomy Farm, Lincoln, NE, in the summers of 1982 and 1983 or in the greenhouse in early 1984. All seedlings analyzed in the laboratory were grown from seed produced either in the field in 1983 or in the greenhouse in early 1984. In total, 52 entries were assayed in the laboratory, including 12 F_1 's, 10 F_2 's, 24 backcrosses, and 6 parental lines (1901, 1904, ARedlan, BRedlan, ACK60, and BCK60).

Twenty-five seed packets were prepared for each of the 52

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entries. Samples of 15 seeds for each parent and F_1 and 20 seeds for each F_2 and backcross were counted, weighed, and placed in separate packets. Seeds produced on sorghum females were free of glumes, but many of the seeds produced on sudangrass females were enclosed in glumes. These glumes were removed in all instances before caryopses were weighed and planted.

All entries were chamber-grown in a randomized complete block design. Planting procedures, seedling culturing, and assays of first leaves of week-old seedlings for HCN-p were essentially as described by Gorz et al. (8). One packet of seed from each entry was included in each of 25 separate plantings, giving 25 replications of the experiment. Two seedlings per replication were assayed for each parental line and F_1 , four for each backcross, and six for each F_2 . This gave a total for the whole experiment of 50 seedlings for each parent and F_1 , 100 for each backcross, and 150 for each F_2 . Sampling in this manner allowed for a greater number of seedlings to be assayed from the segregating generations.

Means and standard errors were computed for each entry by pooling data from the 25 replications. Numbers of seedlings assayed were not equal for all entries as described above; therefore, for each of the 52 entries, means were calculated within each replication, and these means were used in all analyses of variance.

To determine the types of gene effects important in the expression of each trait, generation means analysis was computed by the procedure proposed by Gardner and Eberhart (7). Unweighted data from all entries were analyzed as a diallel set of crosses, and a term was included to test for reciprocal effects (11). Deviations from this model provide an estimate of dominance types of epistasis (7). The relative importance among the genetic effects was determined by dividing the sum of squares for each genetic effect from the generation means analysis by the total sum of squares for all entries involved from the analysis of variance. This calculation gave the fraction of the total variation among entries due to each genetic effect for each trait. These fractions were

multiplied by 100 and reported as percentages of the total sum of squares for each genetic effect.

Heritability estimates in the narrow sense were calculated by the method described by Warner (19) and in the broad sense by Burton's method (3). Phenotypic and genotypic correlations between traits across all entries were determined by using mean squares and variance components from the analyses of variance and covariance as described by Falconer (5). Phenotypic correlations were calculated between seed weight and HCN-p for each entry, each generation, and groups of entries within each generation.

RESULTS AND DISCUSSION

Analysis of variance revealed highly significant differences among entries for both seed weight and seedling HCN-p. Results for the six entries for which seed was produced in the greenhouse did not diverge appreciably from those obtained with field-grown seed; therefore, data obtained with greenhouse- and field-produced seed are considered together.

Seed weights for the sudangrass lines averaged no more than about one-half, and HCN-p values about one-fourth, of the corresponding values for the sorghum parental lines (Table 1). Sudangrass $\times$ sudangrass F_1 's and F_2 's, and backcrosses of the F_1 's to sudangrass parents were generally similar in both seed weight and HCN-p to the sudangrass parents. Also, sorghum $\times$ sorghum F_1 's and backcrosses of these F_1 's to the sorghum parents were similar to the parental lines in seed weight and HCN-p.

Reciprocal crosses between sudangrass and sorghum produced F_1 seed that weighed about the same as seed of the female parents in the crosses (Table 1). Seedling HCN-p values for these F_1 's were intermediate between parental values, and means for sudangrass $\times$ sorghum F_1 's averaged 72% as high as those for

Table 1. Minimum and maximum entry means for seed weight and seedling hydrocyanic acid potential (HCN-p).

Entry group	No. of entries	Seed weight			HCN-p		
		Min.	Max.	LSD(0.05)	Min.	Max.	LSD(0.05)
		mg seed ⁻¹			mg kg ⁻¹ †		
Sudangrass parents	2	9.5	10.7	0.4	249	263	39
Sorghum parents	4	19.3	30.6	0.2	1120	1340	76
F_1 's							
Sudan $\times$ sudan	2	9.1	10.8		274	275	
Sudan $\times$ sorghum	4	11.4	12.8		488	556	
Sorghum $\times$ sudan	4	22.9	26.5		643	777	
Sorghum $\times$ sorghum	4	23.4	28.8		1208	1306	
	12			0.5			57
F_2 's							
Sudan $\times$ sudan	2	10.1	10.9		238	255	
Sudan $\times$ sorghum	4	16.1	19.9		537	616	
Sorghum $\times$ sudan	4	15.4	17.8		536	568	
	10			0.6			37
Backcrosses to sudangrass							
Sudan (sudan $\times$ sudan)	4	9.5	11.8		274	326	
Sudan (sudan $\times$ sorghum)	4	10.5	13.5		402	423	
Sudan (sorghum $\times$ sudan)	4	9.8	13.7		380	415	
	12			0.3			38
Backcrosses to sorghum							
Sorghum (sudan $\times$ sorghum)	4	20.6	32.5		847	985	
Sorghum (sorghum $\times$ sudan)	4	20.8	27.7		876	991	
(Sorghum $\times$ sorghum) sorghum	4	22.3	25.0		1155	1222	
	12			0.6			63

† Fresh weight basis.

Table 2. Mean squares and relative sums of squares for genetic effects from the Gardner and Eberhart (7) generation means analysis.

Source	df	Seed weight		HCN-p	
		Mean square	Sum of squares†	Mean square	Sum of squares†
			%		%
Entry	51	1 087.8**	100	2 921 469**	100
<i>a</i> ‡	3	9 791.5**	53.0	36 948 846**	74.0
<i>hij</i> '	6	1 400.8**	15.2	3 709 456**	15.0
<i>aa_{ij}</i> '	6	252.3	2.7	636 593**	2.6
<i>r_{ij}</i> '	6	314.2**	3.4	87 307**	0.4
Deviation	30	476.6**	25.7	384 943**	8.0
Error	1224	1.0		9 302	

** Significant at the 0.01 level of probability.

† Reported as percentages of the total sum of squares for entry.

‡ *a* = the additive effects due to the *j*th or *j*'th parental line; *hij*' = dominance due to the heterosis effect of the *j*th and *j*'th parental lines; *aa_{ij}*' = the additive by additive interaction effect of the *j*th and *j*'th parental lines; *r_{ij}*' = the reciprocal effect when the *j*th or *j*'th parental line is used as male or female in a cross.

sorghum × sudangrass F_1 's. These differences between reciprocal crosses did not extend into the F_2 generation; thus, it appears that cytoplasmic inheritance was not involved in the differences observed between reciprocal F_1 's.

Backcrosses of the sudangrass × sorghum and sorghum × sudangrass F_1 's produced seed generally similar in size to that of the seed parent in the backcross (Table 1). The HCN-p values for seedlings from backcrosses of these F_1 's to the sudangrass parents were somewhat higher than those of the sudangrass lines. Values for seedlings from backcrosses to the sorghum parents were lower than those for the sorghum parental lines and at least twice as high as those for backcrosses to the sudangrass lines.

The pronounced effect of the female parent on F_1 and backcross seed weight may be due in part to the fact that in the triploid endosperm tissue, two of the three chromosome sets are furnished by the female parent. The pericarp, which is composed of maternal tissue, and the metabolic and transport capabilities of the maternal parent might also influence the size of the caryopsis. Larger seeds should provide correspondingly larger supplies of precursors for dhurrin synthesis, which might help to account for the observed effect of the maternal parent on HCN-p values in the F_1 and backcross generations. As will be seen, however, seed weight and HCN-p were not always closely related to each other.

Frequency distributions of seed weight and HCN-p for the F_2 and backcross generations of the crosses between sorghum and sudangrass did not indicate a clear separation of the progenies into a small number of discrete classes (15). Both traits, therefore, appeared to be inherited quantitatively. The F_1 and F_2 HCN-p distributions, and also F_1 and F_2 mean HCN-p values, were somewhat skewed in the direction of the sudangrass parents, suggesting that in these crosses low HCN-p was partially dominant to high HCN-p.

Variation among entries was used in generation means analysis (7) to ascertain the types of genetic effects that controlled seed weight and HCN-p (Table 2). Additive genetic effects accounted for 53% of the variation for seed weight among entries and genera-

tions. Dominance effects and deviations from the model (dominance types of epistasis) accounted for much of the remaining variation. Voigt et al. (18), investigating the inheritance of seed size in crosses between small- and large-seeded sorghum varieties, found that additive effects accounted for almost all of the variation among generations. Dominance effects were significant in that study also, but accounted for a very small fraction of the variation among generations. Dominance effects and epistasis seemed to play a greater role in the present study.

Additive genetic effects accounted for 74% of the variation for HCN-p among lines and generations (Table 2). The preponderance of additive genetic effects for HCN-p agrees with the findings of Boora and Lodhi (2). Dominance effects accounted for at least half of the remaining variation. Reciprocal effects also were significant for both seed weight and HCN-p, but the fraction of variation attributable to these effects was relatively small (Table 2). A reciprocal effect for HCN-p in the F_1 hybrids was not reported in previous studies. This was probably due to the fact that in most of these studies, either high- or low-HCN-p lines were used consistently as the seed parent (17). Other studies (6, 10) investigated tillers and mature leaves of reciprocal F_1 hybrids but did not reveal a maternal effect on HCN-p. Numerous reports have indicated that HCN-p can be strongly influenced by stage of growth and plant part (e.g., 1, 13, 16). Haskins et al. (13) showed significant positive correlations between seedling and tiller HCN-p. However, it is doubtful that the maternal effect found in week-old seedlings would persist in plant tillers.

Broad- and narrow-sense heritability estimates for HCN-p were 0.60 and 0.42, respectively. Seed weight was recorded only as a mean seed weight per replication; no within-plot error was measured. Therefore, calculations of heritability estimates for seed weight using these methods were not possible in the present study. Heritability estimates for HCN-p probably were maximized by the wide differences in this trait in the chosen parental lines. The observed estimates suggest that progress could be made in breeding for high or low levels of HCN-p. The difference between broad-sense and narrow-sense heritability estimates indicated the importance of nonadditive genetic effects for HCN-p and was in agreement with results previously reported by Gorz et al. (9). Additive × additive epistatic effects for HCN-p were very small in comparison with additive effects (Table 2); therefore, the narrow-sense heritability estimate calculated by Warner's method (19) was not greatly biased.

Phenotypic and genotypic correlations between seed weight and HCN-p across all entries were 0.85** and 0.86, respectively. These correlations reflect the fact that entries with larger seed generally had greater seedling HCN-p. To investigate further the relationship between seed weight and HCN-p, simple correlations between these two traits were calculated for each entry, each generation, and for groups of crosses within each generation. No consistent correlation between seed weight and HCN-p was observed within individual entries. The 52 *r* values ranged from -0.50 to +0.59, and only four were statistically significant. There was

Table 3. Simple correlations between seed weight and hydrocyanic acid potential.

Entries	n	r
All parental lines	150	0.82**
Sudangrass parents	50	0.31*
Sorghum parents	100	-0.07
Reciprocal F ₁ 's	200	0.69**
Sudangrass as seed parent	100	-0.19
Sorghum as seed parent	100	-0.08
Reciprocal F ₂ 's	200	0.11
Sudangrass as seed parent in F ₁	100	0.31**
Sorghum as seed parent in F ₁	100	-0.11
Backcrosses to either sudangrass or sorghum	400	0.85**
Backcrosses to sudangrass	200	0.05
Backcrosses to sorghum	200	0.08

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.

a significant positive correlation between seed weight and HCN-p across all parents (Table 3). This correlation was probably due mainly to the fact that the sudangrass parents, chosen for their low HCN-p, had small seeds while the sorghum parents, chosen for their high HCN-p, had large seeds. No correlation was demonstrated among the sorghum parents, and a significant but low correlation was found among the sudangrass parents, indicating that seed weight had little if any effect on HCN-p within each of the parental types. Significant positive correlations between seed weight and HCN-p were found for all F₁'s considered together and for all backcrosses considered together. However, no significant correlations between seed weight and HCN-p were demonstrated within F₁'s with either sorghum or sudangrass as the seed parent, or in backcrosses to either sorghum or sudangrass alone. Also, no significant relationships between seed weight and seedling HCN-p was indicated by the pooled F₂ data.

In an expanded investigation of the possible relationship of seed size and seedling HCN-p, Lamb (15) used the same six parental lines and eight sorghum-sudangrass F₁'s that were used in the present study. Large and small seeds were selected for each of the 14 entries, and seedling HCN-p values were determined. As in the present study, no significant correlation between seed weight and seedling HCN-p within entries was found. When seeds are identical with respect to the genotypes of both endosperm and embryo, seed weight appears to have little if any relationship to seedling HCN-p.

In summary, quantitative inheritance patterns were observed for both seed weight and seedling HCN-p in crosses between low-HCN-p, small-seeded sudangrasses and high-HCN-p, large-seeded sorghums. Generation means analysis indicated that additive genetic effects were most important in influencing both seed

weight and seedling HCN-p. A maternal effect was observed for both traits in the F₁ and backcross generations, but not in the F₂ generation. Seed weight per se appeared not to have a large effect on seedling HCN-p.

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