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

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

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 F1 and backcross generations. No evidence of this reciprocal effect was found in the F2, suggesting that cytoplasmic inheritance was not involved. A highly positive correlation between seed weight and seedling HCN-p for individual entries, the pooled F2's, or within types of seed parents in the F1 or backcross generations was 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 agreement 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 ms 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 F1 seeds with minimal risk of contamination during crossing. The F1 plants were selfed to produce F2 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 × sorghum F1’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 F1’s, 10 F2’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 entries.
samples of 15 seeds for each parent and F₁, and 20
seeds for each F₂ and backcross were counted, weighed, and
placed in separate packets. Seeds produced on sudangrass fe-
maleenes 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 plant-
ings, giving 25 replications of the experiment. Two seedlings
per replication were assayed for each parental line and F₁,
four for each backcross, and six for each F₂. This gave a total
for the whole experiment of 50 seedlings for each parent and
F₁, 100 for each backcross, and 150 for each F₂. 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 seed-
lings 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 com-
punted 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 cal-
culation 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 cor-
relations 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 dif-
ferences among entries for both seed weight and seed-
ling HCN-p. Results for the six entries for which seed
was produced in the greenhouse did not diverge ap-
preciably 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 × sudangrass F₁’s
and F₂’s, and backcrosses of the F₁’s to sudangrass
parents were generally similar in both seed weight and
HCN-p to the sudangrass parents. Also, sorghum ×
sorghum F₁’s and backcrosses of these F₁’s to the
sorghum parents were similar to the parental lines in
seed weight and HCN-p.

Reciprocal crosses between sudangrass and sorghum
produced F₁ seed that weighed about the same as seed
of the female parents in the crosses (Table 1). Seedling
HCN-p values for these F₁’s were intermediate be-
tween parental values, and means for sudangrass ×
sorghum F₁’s averaged 72% as high as those for

<table>
<thead>
<tr>
<th>Entry group</th>
<th>No. of entries</th>
<th>Seed weight</th>
<th>HCN-p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg seed</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Sudangrass parents</td>
<td>2</td>
<td>9.5</td>
<td>10.7</td>
</tr>
<tr>
<td>Sorghum parents</td>
<td>4</td>
<td>19.3</td>
<td>30.6</td>
</tr>
<tr>
<td>F₁’s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan × sudan</td>
<td>2</td>
<td>9.1</td>
<td>10.8</td>
</tr>
<tr>
<td>Sudan × sorghum</td>
<td>4</td>
<td>11.4</td>
<td>12.8</td>
</tr>
<tr>
<td>Sorghum × sudan</td>
<td>4</td>
<td>22.9</td>
<td>26.5</td>
</tr>
<tr>
<td>Sorghum × sorghum</td>
<td>2</td>
<td>23.4</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>F₂’s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan × sudan</td>
<td>2</td>
<td>10.1</td>
<td>10.9</td>
</tr>
<tr>
<td>Sudan × sorghum</td>
<td>4</td>
<td>16.1</td>
<td>19.0</td>
</tr>
<tr>
<td>Sorghum × sudan</td>
<td>4</td>
<td>15.4</td>
<td>17.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Backcrosses to sudangrass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudan (sudan × sudan)</td>
<td>4</td>
<td>9.5</td>
<td>11.8</td>
</tr>
<tr>
<td>Sudan (sudan × sorghum)</td>
<td>4</td>
<td>10.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Sudan (sorghum × sudan)</td>
<td>4</td>
<td>9.8</td>
<td>13.7</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Backcrosses to sorghum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sorghum (sudan × sorghum)</td>
<td>4</td>
<td>20.6</td>
<td>32.5</td>
</tr>
<tr>
<td>Sorghum (sorghum × sudan)</td>
<td>4</td>
<td>20.8</td>
<td>27.7</td>
</tr>
<tr>
<td>(Sorghum × sorghum) sorghum</td>
<td>4</td>
<td>22.3</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

† Fresh weight basis.
Backcrosses of these FI's to the sudangrass parents were and backcross seed weight may be due in part to the lines and at least twice as high as those for backcrosses parents were lower than those for the sorghum parental lines. The pronounced effect of the female parent on F₁ 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 HCN-p that are present 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 2. Mean squares and relative sums of squares for genetic effects from the Gardner and Eberhart (7) generation means analysis.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>Sum of squares†</th>
<th>Mean square</th>
<th>Sum of squares†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry</td>
<td>51</td>
<td>1 087.8**</td>
<td>100</td>
<td>2 921 469**</td>
<td>100</td>
</tr>
<tr>
<td>s²f</td>
<td>3</td>
<td>9 791.5**</td>
<td>53.0</td>
<td>36 948 846**</td>
<td>74.0</td>
</tr>
<tr>
<td>aij'</td>
<td>6</td>
<td>1 450.0**</td>
<td>15.2</td>
<td>7 309 456**</td>
<td>16.0</td>
</tr>
<tr>
<td>sij'</td>
<td>6</td>
<td>222.3</td>
<td>2.7</td>
<td>636 893**</td>
<td>2.8</td>
</tr>
<tr>
<td>rif</td>
<td>6</td>
<td>31.4**</td>
<td>3.4</td>
<td>87 307**</td>
<td>0.4</td>
</tr>
<tr>
<td>Deviation</td>
<td>80</td>
<td>476.8**</td>
<td>25.7</td>
<td>864 949**</td>
<td>8.0</td>
</tr>
<tr>
<td>Error</td>
<td>1224</td>
<td>1.0</td>
<td>9 302</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** 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 jth or j'th parental line; aij' = dominance due to the heterosis effect of the jth and j'th parental lines; sij' = the additive by additive interaction effect of the jth and j'th parental lines; rif = the reciprocal effect when the jth or j'th parental line is used as male or female in a cross.

### Sorghum × sudangrass F₁'s. These differences between reciprocal crosses did not extend into the F₂ generation; thus, it appears that cytoplasmic inheritance was not involved in the differences observed between reciprocal F₁'s.

Backcrosses of the sudangrass × sorghum and sorghum × sudangrass F₁'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 F₁'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 F₁ 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 F₁ 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 F₂ 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 F₁ and F₂ HCN-p distributions, and also F₁ and F₂ 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-

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

<table>
<thead>
<tr>
<th>Entries</th>
<th>n</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>All parental lines</td>
<td>150</td>
<td>0.82**</td>
</tr>
<tr>
<td>Sudangrass parents</td>
<td>50</td>
<td>0.31*</td>
</tr>
<tr>
<td>Sorghum parents</td>
<td>100</td>
<td>-0.07</td>
</tr>
<tr>
<td>Reciprocal F₁'s</td>
<td>200</td>
<td>0.69**</td>
</tr>
<tr>
<td>Sudangrass as seed parent</td>
<td>100</td>
<td>-0.19</td>
</tr>
<tr>
<td>Sorghum as seed parent</td>
<td>100</td>
<td>-0.08</td>
</tr>
<tr>
<td>Reciprocal F₁'s</td>
<td>200</td>
<td>0.11</td>
</tr>
<tr>
<td>Sudangrass as seed parent in F₂</td>
<td>100</td>
<td>0.31**</td>
</tr>
<tr>
<td>Sorghum as seed parent in F₂</td>
<td>100</td>
<td>-0.11</td>
</tr>
<tr>
<td>Backcrosses to either sudangrass or sorghum</td>
<td>400</td>
<td>0.86**</td>
</tr>
<tr>
<td>Backcrosses to sudangrass</td>
<td>200</td>
<td>0.06</td>
</tr>
<tr>
<td>Backcrosses to sorghum</td>
<td>200</td>
<td>0.08</td>
</tr>
</tbody>
</table>

** 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- and high-HCN-p, small- and 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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