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ABSTRACT

Grain sorghum [*Sorghum bicolor* (L.) Moench] typically produces larger seeds than sudangrass [*S. bicolor*, formerly *S. sudanense* (Piper) Stapf]; and grain sorghum seedlings are higher in hydrocyanic acid potential (HCN-p) than sudangrass seedlings. Previous studies have shown a seed-parent effect on seed weight and HCN-p in reciprocal F_1 hybrids of sorghum × sudangrass. This study was conducted to determine whether the seed-parent effect on HCN-p could be attributed primarily to the difference in seed size between reciprocal sorghum × sudangrass hybrids. Large and small seeds of low–HCN-p sudangrass, high–HCN-p sorghum, and their reciprocal hybrids, were visually selected, individually weighed, and planted in growth chambers. Height, fresh weight, and HCN-p of the resulting 7-d-old shoots were measured. On average, the selected large seeds weighed ≈ 1.6 times as much as the small seeds. Shoots from the large seeds were ≈ 1.2 times as tall and 1.4 times as heavy as those from small seeds, and they contained ≈ 1.3 times as much HCN per shoot as did shoots from small seeds. However, the HCN-p (mg kg^{-1} fresh wt.) of the shoots from large seeds was only slightly greater than that of shoots from small seeds (709 and 701 for first leaves of shoots, and 278 and 267 for shoot remainders from large and small seeds, respectively). Shoots from 5-d-old light-grown and etiolated seedlings also failed to show appreciable differences in HCN-p due to seed size. It was concluded that the seed-parent effect on shoot HCN-p in crosses of sorghum and sudangrass was not caused primarily by the seed-parent effect on seed weight.

In a recent study of the inheritance of seedling hydrocyanic acid potential in crosses between high–HCN-p sorghum and low–HCN-p sudangrass, results indicated a maternal or seed-parent effect for seed weight and seedling HCN-p in the F_1 and backcross generations (Lamb et al., 1987); however, no evidence of this reciprocal effect was found in the F_2, suggesting that cytoplasmic inheritance is not involved. High, positive correlations ($P \leq 0.01$) between seed weight and seedling HCN-p were found for all entries taken together ($r = 0.85^{**}$; significant at $p \leq 0.01$), for all parental lines ($r = 0.82^{**}$), for all F_1 populations ($r = 0.69^{**}$), and for all backcross populations ($r = 0.85^{**}$) (Lamb et al., 1987); however, correlations between seed weight and seedling HCN-p for individual entries, the pooled F_2's, or within types of seed parent in the F_1 or backcross generations were generally nonsignificant. Thus, seed weight per se appeared not to have a large effect on seedling HCN-p.

The foregoing report (Lamb et al., 1987) dealt only with seed weight and seedling HCN-p, and values for seed weight were means calculated from the number and total weight of seeds planted in each replicate. Weights of individual seeds giving rise to the sampled seedlings were not known. The primary objective of the present study was to investigate in greater detail the possible effect of seed weight on seedling HCN-p and other seedling traits within sorghum and sudangrass parental lines and reciprocal F_1 sorghum × sudangrass hybrids. Backcross and F_2 populations were excluded because the genetic diversity in such populations was undesirable for this study. Individual seeds were weighed, and measurements were made of the fresh weight, height, and HCN-p of individual shoots arising from identified seeds.

A brief study also was made that used seedlings grown in water-saturated vermiculite. The rationale for this study was that in the absence of exogenous nutrients, seed-size dependent differences in seedling traits might be especially distinct.

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Abbreviation: HCN-p, hydrocyanic acid potential.

MATERIALS AND METHODS

Seven-Day, Light-Grown Seedlings

Two low–HCN-p sudangrass lines, 1901 and 1904; and four high–HCN-p sorghum lines, ACK60, BCK60, ARedlan, and BRedlan, were used as parental lines in this study. Reciprocal sorghum × sudangrass hybrids included the following: 1901 × BCK60, ACK60 × 1901, 1901 × BRedlan, ARedlan × 1901, 1904 × BCK60, ACK60 × 1904, 1904 × BRedlan, and ARedlan × 1904. Further descriptions of the parental lines and the crosses used to produce the F_1 hybrids are given by Lamb et al. (1987).

Large and small seeds of each entry were selected visually. For each replicate, 10 large and 10 small seeds of each entry were individually weighed. The 10 large seeds of a given entry were planted in identified positions in a single row in a small plastic pan, and 10 small seeds of the same entry were planted in an adjacent row. The 14 entries (14 pairs of rows) were randomly arranged in each replicate of the experiment, and eight separate plantings provided a total of eight replicates. Seedlings were grown in a soil mixture under continuous cool-white fluorescent light (≈ 150 μmol m^{-2} s^{-1}) at 27° C, essentially as described by Gorz et al. (1977).

At 7 d after planting, three representative seedlings were chosen for assay from each of the 28 rows. Seedling height was measured, and the first leaf of each sampled seedling was then harvested separately from the shoot remainder, which was excised at the soil surface. Both shoot portions were weighed, extracted, and assayed as described by Gorz et al. (1977).

Analysis of variance for a randomized complete-block design was used for all traits measured. The error cross products and sums of squares matrix from this analysis were used to calculate partial correlation coefficients for seedlings within entries for all traits. Phenotypic correlations between traits across all entries were determined by using mean squares and mean products from the analyses of variance and covariance as described by Falconer (1981).

Five-Day, Light- and Dark-Grown Seedlings

Parental lines 1904 and BRedlan and the crosses 1904 × BRedlan and ARedlan × 1904 were used. Two sets of 20 small and 20 large seeds (individually weighed) of each parent and F_1 hybrid were planted in water-saturated vermiculite. One set was placed in continuous light at 27° C as described above, and the other in a light-excluding container in the same growth chamber. At 5 d, seedlings were harvested and each was separated into shoot, root, and seed remnant. The portions were bulked within entry, light treatment, seed size, and type of portion. The bulked samples were weighed, extracted, and assayed for HCN-p by the spectrophotometric procedure of Gorz et al. (1977). Also, the absorbance spectrum of each base-diluted extract was scanned from 400 to 220 nm.

RESULTS AND DISCUSSION

Seven-Day, Light-Grown Seedlings

Results were similar within each of the parental types and also within each type of F_1 hybrid; therefore, data were pooled within each of these four types of entry as shown in Table 1. Means for both first-leaf and shoot-remainder HCN-p showed the same sort of seed-parent influence as that previously observed by Lamb et al. (1987); that is, values for sorghum × sudangrass hybrids were higher than those for sudangrass × sorghum hybrids. This was true of seedlings from both small and large seeds. Results were consistent within entries and across all entries for seed size differences (Table 1). Seedlings from large seeds were significantly taller and had significantly greater first-leaf and shoot-remainder weights than those from small seeds. There were no significant differences due to seed size within entries or across all entries for first-leaf HCN-p or shoot-remainder HCN-p, except for shoot-remainder HCN-p of sudangrass × sorghum seedlings, and that difference was small (12 mg kg^{-1} fresh wt.). Hydrocyanic acid per shoot is a product of shoot weight and HCN-p, and the apparent effect of seed size on HCN per shoot was primarily a result of the effect on shoot weight rather than on HCN-p.

Within-entry and overall phenotypic correlations for all pairs of traits are shown in Table 2. The within-entry values indicated significant association of seed

Table 1. Influence of seed size on various traits of 7-d-old light-grown seedlings of low–HCN-p (hydrocyanic acid-potential) sudangrasses, high-HCN-p sorghums, and their reciprocal F_1 hybrids.†

Trait	Seed size	Sudangrass parents	Sorghum parents	Sudangrass × sorghum	Sorghum × sudangrass	Over all entries
Seed weight,	Small	7.1 ± 0.1**	18.9 ± 0.4**	8.2 ± 0.1**	17.7 ± 0.3**	13.8 ± 0.3**
mg $seed^{-1}$	Large	12.5 ± 0.1	27.6 ± 0.5	14.7 ± 0.1	27.9 ± 0.2	21.8 ± 0.4
Seedling height,	Small	111 ± 2**	87 ± 1**	113 ± 1**	134 ± 2**	112 ± 1**
mm	Large	133 ± 4	97 ± 1	141 ± 1	150 ± 2	130 ± 1
First-leaf weight,	Small	13.5 ± 0.4**	12.5 ± 0.3**	14.8 ± 0.2**	16.5 ± 0.3**	14.5 ± 0.2**
mg fr wt.	Large	16.6 ± 0.5	13.6 ± 0.2	19.0 ± 0.3	19.2 ± 0.3	17.2 ± 0.2
First-leaf HCN-p,	Small	221 ± 10	1194 ± 24	487 ± 9	674 ± 18	701 ± 21
mg kg^{-1} fr wt.	Large	226 ± 10	1167 ± 22	505 ± 10	695 ± 13	709 ± 19
Shoot–remainder weight,	Small	38.4 ± 1.4**	66.5 ± 2.3**	50.7 ± 1.1**	93.0 ± 2.3**	65.5 ± 1.5**
mg fr wt.	Large	56.2 ± 2.7	83.0 ± 2.3	92.1 ± 1.7	131.1 ± 2.5	95.5 ± 1.8
Shoot–remainder HCN-p,	Small	94 ± 4	516 ± 10	147 ± 3*	229 ± 7	267 ± 10
mg kg^{-1} fr wt.	Large	101 ± 4	518 ± 10	159 ± 4	244 ± 6	278 ± 9
Total shoot weight,	Small	51.9 ± 1.7**	79.0 ± 2.5**	65.4 ± 1.2**	109.4 ± 2.6**	80.0 ± 1.6**
mg fr wt.	Large	72.7 ± 3.1	96.6 ± 2.4	111.1 ± 1.9	150.3 ± 2.7	112.7 ± 1.9
Total HCN $shoot^{-1}$,	Small	6.6 ± 0.3**	49.3 ± 1.6**	14.6 ± 0.3**	31.8 ± 0.9**	28.2 ± 1.0**
μg	Large	9.5 ± 0.5	59.0 ± 1.7	24.0 ± 0.5	44.5 ± 0.8	37.8 ± 1.1

*,** Differences between the two seed sizes are significant at the 0.05 and 0.01 levels of probability, respectively, as shown by a *t*-test.
† Means and standard errors are shown; $n = 48$ for sudangrass parents, 336 for all entries combined, and 96 for the other entries. Fresh weight is denoted by fr wt.

Table 2. Partial correlation coefficients for seedlings of sudangrass and sorghum and their reciprocal F_1 hybrids within entries (above diagonal) and overall phenotypic correlations for pairs of traits of all entries (below diagonal).

	Seed weight	Seedling height	First-leaf weight	First-leaf HCN-p†	Shoot-remainder weight	Shoot-remainder HCN-p	Total shoot weight	Total HCN per shoot
Seed weight	–	0.56**	0.47**	0.06	0.70**	0.12**	0.69**	0.57**
Seedling height	−0.18	–	0.76**	−0.04	0.81**	−0.08	0.82**	0.51**
First-leaf weight	−0.15	0.89**	–	−0.17**	0.77**	0.13**	0.81**	0.53**
First-leaf HCN-p	0.73**	−0.67**	−0.50	–	0.00	0.70**	−0.02	0.40**
Shoot-remainder weight	0.69**	0.52	0.53*	0.24	–	0.00	0.99**	0.76**
Shoot-remainder HCN-p	0.70**	−0.76**	−0.59*	0.98**	0.12	–	−0.01	0.49**
Total shoot weight	0.64*	0.58*	0.60*	0.18	0.99**	0.06	–	0.75**
Total HCN $shoot^{-1}$	0.88**	−0.47	−0.32	0.95**	0.48	0.93**	0.42	–

*,** Significant at the 0.05 and 0.01 levels of probability, respectively.
† HCN-p = hydrocyanic acid potential.

weight with each of the seedling height and weight traits, as well as between seed weight and total HCN per shoot. However, the association between seed weight and first-leaf HCN-p was nonsignificant, and that between seed weight and shoot-remainder HCN-p, while statistically significant, was not close (r = 0.12). The various seedling weight and height traits were significantly associated with each other and with seed weight, but none was closely associated with the HCN-p traits. The closest association was observed for the relationship of total shoot weight and shoot-remainder weight (r = 0.99). This result was not surprising inasmuch as the shoot remainder accounted for an average of > 80% of the total shoot weight. Total HCN per shoot was significantly correlated with each of the other measured traits, reflecting the dependence of total HCN on both the HCN-p and the quantity of shoot tissue.

When all entries were considered together (overall phenotypic values, Table 2) a strong relationship between seed weight and HCN-p was apparent. As pointed out by Lamb et al. (1987) and as verified by the data in Table 1, this may be attributed to the fact that the sorghum entries had larger seeds and higher seedling HCN-p values than the sudangrass entries. Also, seeds from the sudangrass × sorghum crosses were smaller and seedlings were lower in HCN-p than corresponding seeds and seedlings from sorghum × sudangrass hybrids. The mean comparisons discussed previously and the correlations within and across entries all verify the previous research of Lamb et al. (1987), which indicated that when seeds are identical with respect to genotype of both endosperm and embryo, seed weight has little if any effect on seedling HCN-p. Therefore, it is reasonable to infer than the seed-parent effect on seedling HCN-p in crosses of sorghum and sudangrass was not due primarily to the seed-parent effect on seed weight.

Five-Day, Light- and Dark-Grown Seedlings

Unfortunately, germination varied greatly in this test, and the number of available seedlings varied from 6 to 20 seedlings among the treatments. Also, although the spectral scans revealed a clear 330-nm peak in each of the shoot extracts, the extracts of roots and seed remnants contained substances that interfered seriously with the reading at 330 nm, the wavelength on which the spectrophotometric assay depends (Gorz et al., 1977). Despite these difficulties, several observations of interest resulted from this experiment.

1. The shoot portion accounted for 37 ± 3% (mean ± SE) of the fresh w and 82 ± 1% of the HCN content of 5-d light-grown seedlings. Corresponding averages for etiolated seedlings were 61 ± 4% and 90 ± 2%. The observation that a preponderance of the seedling's HCN was present in the shoot agrees with the reports of Akazawa et al. (1960) and Loyd and Gray (1970).
2. The seed-parent effect on HCN-p, and differences in HCN-p, were less distinct for 5-d than for 7-d light-grown seedlings, and no seed-parent effect was observed in 5-d etiolated shoots. Respective HCN-p values for 5-d light-grown shoots of 1904, BRedlan, 1904 × BRedlan, and ARedlan × 1904 were 481, 1362, 803, and 929 mg kg^{-1} fresh wt. Corresponding values for 5-d etiolated shoots were 293, 374, 358, and 367 mg kg^{-1} fresh wt. It appears that seedlings must be grown in the light for more than 5 d for differences in HCN-p to be fully expressed.
3. Large/small seed weight ratios were 1.69 ± 0.11 for the light-grown seedlings and 1.68 ± 0.08 for the etiolated seedlings. Corresponding ratios for shoot HCN-p were 1.08 ± 0.06 and 1.03 ± 0.03. As in the case of 7-d, soil-grown seedlings, seed size had little effect on shoot HCN-p.

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