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NOTES

SOURCES OF VARIATION IN THE SPECTROPHOTOMETRIC ASSAY OF HYDROCYANIC ACID POTENTIAL IN SORGHUM SEEDLINGS

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Abstract

Spectrophotometry is a useful assay for hydrocyanic acid potential (HCN-p) in sorghum and sudangrass [both *Sorghum bicolor* (L.) Moench] seedlings, but no systematic study of sources of variation in the procedure has been reported. Selfed seed was harvested from each of 12 ramets (two each from two sister plants from three low-HCN-p sudangrass parents), and seedlings for sampling were grown in a growth chamber, in two rows from each ramet. Seven-day-old seedlings were harvested and divided into two samples per row for extraction; two aliquots per extract were assayed spectrophotometrically for HCN-p. The experiment was replicated three times. The three parents differed in HCN-p. Rows within ramets, and samples within rows, were also significant sources of variation, but the magnitude of variance component estimates was small relative to that of the parents. Sister plants within parents, ramets within plants, and aliquots within samples were not significant sources of variation.

A SIMPLE, rapid, and nondestructive spectrophotometric method (2) has been used for a number of years for assay of hydrocyanic acid potential (HCN-p) of young sorghum and sudangrass seedlings. The procedure is based on determination of *p*-hydroxybenzaldehyde (*p*-HB) released from the hydrolysis of dhurrin [*p*-hydroxy-(*S*)-mandelonitrile-β-D-[glucoside], the cyanogenic compound of sorghum, when seedling leaves are autoclaved in water. Because dhurrin hydrolysis produces HCN and *p*-HB in equimolar amounts, HCN-p values can be calculated readily from absorbance values for *p*-HB.

The spectrophotometric procedure has been used successfully in studies on influence of mineral elements (1) and radiation (7) on the HCN-p of sorghum seedlings. This procedure has been useful in investigations on inheritance of seedling HCN-p (8) and in identifying those sorghum chromosomes that have a significant influence on seedling HCN-p (4). Divergent selection for seedling HCN-p in 'Greenleaf' sudangrass was demonstrated by use of this technique (5), and a total of 19 sudangrass (e.g., Ref. 6) and six sorghum (e.g., 3) lines and populations with lowered seedling HCN-p have been developed, released, and registered.

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Although the spectrophotometric procedure has been used with apparent success, a systematic investigation of sources of variation in the procedure has not been reported previously. This report presents results of such an investigation.

Materials and Methods

Two sister plants from each of three low-HCN-p sudangrass parents were used. The six plants were grown in the greenhouse during the winter, and in the following spring the crown of each plant was separated into several ramets for transplanting to the field at Lincoln, NE. Selfed seed was harvested from each ramet. Subsequent seedling assays were confined to two ramets from each plant (total of 12 ramets) and to seed harvested from panicles that were bagged within a 3-d period in July.

Seedlings for assay were grown in plastic trays of soil in a growth chamber at 27 °C under continuous cool-white fluorescent light at ≈150 μmol photon m^{-2} s^{-1}, essentially as described by Gorz et al. (2). Two rows were planted from each ramet, with 45 seed row^{-1}, and the 24 rows were arranged at random in trays of soil. First seedling leaves were harvested 7 d after planting. Where possible, 20 uniform leaves were harvested from each row, and these leaves were allocated at random into two 10-leaf samples. For rows from which fewer than 20 suitable leaves were obtained, an even number of leaves were harvested and divided at random into two samples. Weighed samples were autoclaved in water to extract and hydrolyze dhurrin, and extracts were diluted in 0.1 *M* NaOH for reading absorbance values at 330 nm, the absorbance maximum of *p*-HB (2). Two aliquots of each extract were diluted and recorded.

With 3 parents, 2 plants $parent^{-1}$, 2 ramets $plant^{-1}$, 2 rows $ramet^{-1}$, 2 samples row^{-1}, and 2 aliquots $sample^{-1}$, there were 96 readings per replication. Plantings made on three separate dates provided three replications of the experiment, for a total of 288 readings.

For analysis of variance, parents were assumed to be fixed sources of variation, while plants nested within parents, ramets nested within plants, rows nested within ramets, samples nested within rows, and aliquots nested within samples were assumed to be random sources of variation. Therefore, the error terms for *F*-tests involving parents, plants within parents, ramets within plants, rows within ramets, samples within rows and aliquots within samples were, respectively: plants within parents, ramets within plants, rows within ramets, samples within rows, aliquots within samples, and mean square error. Statistical analysis were calculated using SAS (9).

Results and Discussion

Analysis of variance (Table 1) revealed that plants, ramets, and aliquots were not significant sources of variation in the assay of the three parental sources. The parent source of variation, however, was significant ($P \leq 0.05$). Least square mean values for the three parents were 420, 350, and 260 mg kg^{-1} fresh wt., with standard errors of 6.0, 6.0, and 6.2 mg kg^{-1} fresh wt., respectively.

Row and sample sources of variation also were significant ($P \leq 0.01$). The basis for the difference between rows is not immediately obvious; rows were randomly arranged in the growth chamber, and systematic differ-

Abbreviations: HCN-p, hydrocyanic acid potential; *p*-HB, *p*-hydroxybenzaldehyde.

Table 1. Analysis of variance for hydrocyanic acid potential (HCN-p) of three sudangrass parents with five levels of subsampling.

Source	df	Mean squares	*F*-value
No. plants per sample (Sp)†	1	10 805	
Replications	2	137 840	
Parents (Pr)	2	555 448	15.35*
Plant (Pt) / Pr	3	36 193	4.17
Ramet (Rt) / Pr Pt	6	8 674	0.87
Row (Rw) / Pr Pt Rt	12	10 022	4.65**
Sample (Sp) / Pr Pt Rt Rw	24	2 156	30.16**
Aliquot / Pr Pt Rt Rw Sp	48	72	0.02
Error	189	3 371	
Total	287		

*, ** Significant at the 0.05 and 0.01 probability levels, respectively.
† Included as a covariate, since samples (Sp) did not always consist of 10 uniform leaves.

ences due to soil or light conditions do not seem likely. Similarly, the basis for the significant difference between samples within rows also is not completely obvious; however, contributing factors probably include true differences in HCN-p of the two samples taken from each row, differences in extraction and hydrolysis of dhurrin during autoclaving of the samples, and differences in evaporation of the extracts during and immediately following autoclaving. Nevertheless, these sources of significant variation were not of sufficient magnitude to preclude observing significant differences among parents. Indeed, comparison of variance component estimates for parent ($\hat{\sigma}^2_{Pr}$ = 57 696), row ($\hat{\sigma}^2_{Rw}$ = 656), and sample ($\hat{\sigma}^2_{Sp}$ = 347) reveals that the parent component possesses the overwhelming proportion of the total variation. The aliquot source of variation, with an extremely low mean square and *F*-value, indicates that once an extract was prepared, excellent precision in diluting and in reading absorbance values was achieved.

Variation of the type observed in this brief investigation detracts from the efficiency of the procedure in attempts to classify seedlings according to HCN-p level and does identify stages in the HCN assay procedure where attention to laboratory technique is critical. In spite of these unexplainable sources of variation, however, the procedure did successfully differentiate HCN-p among parents and, as has been noted, the procedure has been used successfully in selection programs (3,5,6).

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