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DHURRIN AND *p*-HYDROXYBENZALDEHYDE IN SEEDLINGS OF VARIOUS *SORGHUM* SPECIES*

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Key Word Index—*Sorghum* species; Gramineae; dhurrin; *p*-hydroxybenzaldehyde; cyanogenesis.

Abstract—Week-old shoots of 50 *Sorghum* entries representing 22 species, plus four *Sorghum* entries of undesignated species, were dried at 75° and the dried tissue extracted with water at room temperature. The resulting extracts were diluted in 0.1 M sodium hydroxide and spectra were scanned immediately to provide a measure of free *p*-hydroxybenzaldehyde. Scans were repeated after the basic solutions had stood for 3 hr at room temperature to permit hydrolysis of dhurrin (*S*-*p*-hydroxymandelonitrile β-D-glucopyranoside). Without exception, the quantity of free *p*-hydroxybenzaldehyde was very small in relation to the quantity released by dhurrin hydrolysis.

Woodhead *et al.* [1] observed that free *p*-hydroxybenzaldehyde (**1**) makes up as much as 30% of the wax of *Sorghum bicolor* seedlings. This observation raised doubts as to the validity of a dhurrin (*S*-*p*-hydroxymandelonitrile β-D-glucopyranoside) assay that we have used extensively. In this assay *Sorghum* seedling tissue is autoclaved in water to extract and simultaneously hydrolyse the dhurrin, and the liberated **1** is determined spectrophotometrically to provide a measure of dhurrin content [2]. Following the report of Woodhead *et al.* [1], we did a series of experiments with several *Sorghum* cvs. from which we concluded that free **1** was not present in appreciable quantity on the surface or within young shoots that had been heated to inactivate hydrolytic enzymes [3]. Subsequently, Woodhead *et al.* [4] have stated that, although the occurrence of free **1** in *Sorghum* seedlings is "clearly a restricted phenomenon with many cultivars having little or no free **1**", seedlings of cv. 65D from Botswana and one other cultivar, not identified, contained **1** in the surface wax.

The possibility that free **1** might occur in seedlings of some but not all *Sorghums* led us to the experiments reported here in which we have examined seedlings of a diverse group of 54 *Sorghum* accessions for the presence of this compound.

RESULTS AND DISCUSSION

Extracts from shoots of 1-week-old seedlings of each sample (dried at 75° for 2.5 hr) were made in water and diluted with 0.1 M sodium hydroxide. Each basified extract was scanned from 400 to 240 nm immediately after dilution, and scans were repeated after the solutions had stood at room temperature for *ca* 3 hr to allow hydrolysis of dhurrin.

All initial spectral scans were similar in that each lacked a well-defined peak at 330 nm (the UV λ_{max} of **1**) but had a definite peak at 255 nm (the UV λ_{max} of dhurrin in alkaline solution). After 3 hr, each scan had a strong 330 nm peak and had lost the 255 nm peak. Based on the A_{330} values obtained, the total concentration of **1** for all 54 entries (mean ± s.e.) was 19.6 ± 1.4 μmols/g fr. tissue.

For most entries, the increase in A_{330} accompanying the basic hydrolysis of dhurrin was greater than 10-fold; the mean A_{330} (initial)/A_{330} (3 hr) ratio being 0.092 ± 0.005. All initial scans were relatively flat in the region between 400 and 320 nm. This flatness is illustrated by the A_{380}/A_{330} ratio which was 1.03 ± 0.03 for initial scans, in contrast to 0.099 ± 0.005 for 3 hr scans.

It was clear from the spectral scans that interference with the A_{330} maximum of **1** was relatively much greater in the initial scans than in those made at 3 hr and that use of the initial A_{330} readings for calculation of the concentration of free **1** would lead to highly inflated values. An indication of the extent of this inflation is provided by other work with 10 cultivars and lines of *S. bicolor*, one of which was 65D. Dried, ground 1-week-old shoots were extracted with either chloroform or, in some instances, with water followed by ether extraction of the aqueous extracts. Scans of the chloroform and ether extracts indicated that less than 1% of the total content of **1** was present in the free form ([3] and unpublished results). Thus, the true ratio of free to total **1** is probably closer to 0.01 than to the 0.092 value shown above. We conclude that little, if any, free **1** existed within or on shoots of any of the entries included in this study.

With respect to content of free **1** we found cv. 65D to be similar to all other *Sorghums* included in the study. We are unable to explain the lack of agreement between our results and those of Woodhead *et al.* [4] with this cultivar.

EXPERIMENTAL

Plant materials. Seeds of 53 entries were obtained from the U.S. Department of Agriculture Regional Plant Introduction Station,

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Experiment, Georgia. Included were one accession of *Sorghum aethiopicum*, three of *almum*, three of *arundinaceum*, three of *bicolor*, four of *caudatum*, one of *controversum*, one of *halepense*, two of *hewisonii*, one of *japonicum*, one of *miliaceum*, three of *nigricans*, one of *niloticum*, three of *notabile*, one of *plumosa*, one of *propinquum*, one of *pugionifolium*, four of *saccharatum*, two of *subglabrescens*, seven of *sudanense*, one of *versicolor*, three of *verticilliflorum*, two of *virgatum* and four accessions designated only as *Sorghum* sp. In addition, cv. 65D (*S. bicolor*) was obtained from L. M. Mazhani, Department of Agricultural Research, Gaborone, Republic of Botswana. In total, 50 entries representing 22 species plus four entries without species designation were included in the study. Seedlings were grown as previously described [2]. Samples usually consisted of a bulk of five shoots from 1-week-old seedlings.

Sample treatment and spectral scanning. Samples were weighed, dried at 75° for 2.5 hr, pulverized and extracted with 20 ml H_2O at room temp. for 2 hr. The tissue residue was removed by filtration and filtrates were diluted 10-fold with 0.1 M NaOH for spectral scanning.

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