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Short communication

Relationship between contents of leucoanthocyanidin and dhurrin in sorghum leaves*

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Summary. Flag leaves of 'Colman' forage sorghum (*Sorghum bicolor*) contain at least 25 times as much leucoanthocyanidin (LAC) and approximately half as much of the cyanogenic glucoside, dhurrin, as do flag leaves of 'White Collier' forage sorghum. Assays of flag leaves from 119 F_2 plants and 11 F_5 lines from crosses between these two cultivars revealed a statistically significant negative association between levels of LAC and dhurrin. Both LAC and dhurrin are aromatic compounds, and the negative association between the two may be the result of competition for intermediates or products of the aromatic biosynthetic pathway. This rationale appears to be quite different from that for the negative association reported for levels of tannin and cyanide in *Lotus corniculatus.* Although the negative relationship between LAC and dhurrin in sorghum was statistically significant, the association was not consistent enough to suggest that either trait could be used reliably in selecting or breeding to modify the other trait.

Key words: Cyanogenesis – Dhurrin – Leucoanthocyanidin – *Sorghum bicolor*

Introduction

Ross and Jones (1983) reported a strong negative association between contents of tannin and cyanide in *Lotus corniculatus* leaves harvested from six cultivars with different levels of leaf tannins. Sorghum (*Sorghum bicolor*) leaves probably do not contain tannins (polymeric proanthocyanidins), but leaves of certain cultivars do contain monomeric leucoanthocyanidins (LAC's) that are structurally related to tannins (Watterson and Butler 1983). The LAC content of 'Colman' (LAC+) sorghum flag leaves is at least 25 times as great as that of 'White Collier' (LAC–) flag leaves, and this large difference is controlled primarily by a single allelic pair (Haskins and Gorz 1986). Sorghum leaves also contain the cyanogenic glucoside, dhurrin (*p*-hydroxy-(S)-mandelonitrile-β-D-glucoside), and preliminary assays in our laboratory indicate that the dhurrin concentration of 'Colman' flag leaves is about half as great as that of 'White Collier' flag leaves. This difference has not been studied in detail, however, and its possible genetic control has not been elucidated.

The purpose of the work reported in this paper was to determine whether a negative association might exist between LAC and dhurrin in sorghum resembling the reported relationship between tannin and cyanide in *L. corniculatus.* The existence of such a relationship in sorghum would be of interest from the standpoint of the metabolism of LAC and dhurrin, and might also be useful in attempts to breed for altered levels of either of these constituents.

Materials and methods

Reciprocal crosses between the forage sorghum [*Sorghum bicolor* (L.) Moench] cultivars, 'Colman' and 'White Collier', were made by hand emasculation and pollination, and F_1 plants were self-pollinated for the production of F_2 seed. In 1984, 119 F_2 plants were grown in the field at the University of Nebraska Agricultural Research and Development Center, Mead, Nebraska. Samples of the blade of the flag leaf from the main stem of each plant were harvested at head emergence, midribs were removed and discarded, and the remaining tissue was dried at 75 °C, ground through a 1-mm screen, and held in a freezer prior to extraction for assay of LAC and dhurrin.

The procedures and results of the LAC assay of these samples have been reported (Haskins and Gorz 1986). The

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absorbance of acidified methanolic extracts at 540 nm was used as a measure of LAC in this assay.

The procedure of Lambert et al. (1975) as modified by Gorz et al. (1986) was used for assaying dhurrin content. In this procedure, dhurrin was hydrolyzed in 0.1 M NaOH, and the cyanide in the hydrolysate was measured colorimetrically. Results were expressed as HCN potential (HCN-p) in mg/kg dry tissue.

Another experiment employed 11 F_5 lines derived from the crosses between 'Colman' and 'White Collier'. Six of these lines were homozygous LAC–; the other five were homozygous LAC+. A 9-m row of each line was grown in the field in 1985, and at head emergence the upper four or five leaf blades were harvested from each stem. The harvested leaves were bulked within lines. Midribs were discarded, and the remaining tissue was dried, ground, and stored in a freezer as in 1984. Portions of the 11 ground preparations were extracted for assay of LAC and dhurrin.

Results

As previously reported (Haskins and Gorz 1986), the 119 F_2 plants grown in 1984 segregated for LAC content. Eighty-seven plants were classified as LAC+ with an A_{540} mean ± SE of 0.268 ± 0.019, and 32 plants as LAC– with 0.032 ± 0.002. Overall the mean A_{540} value was 0.204 ± 0.017, and the range was 0.016 to 0.85. The corresponding mean HCN-p was 195 ± 11 mg kg^{-1}, and the range was 73 to 607. The r value for the relationship between the two variables was –0.42, which exceeds the value required for significance at the 0.01 probability level.

The 11 preparations from the 1985 harvest ranged from 0.011 to 1.03 in A_{540} and from 104 to 426 mg kg^{-1} in HCN-p. Values for A_{540} and HCN-p, respectively, for the five LAC+ lines averaged 0.728 ± 0.102 and 134 ± 8; for the six LAC– lines the means were 0.015 ± 0.001 and 279 ± 37. The r value for the relationship between A_{540} and HCN-p was –0.68, which is significant at the 0.05 probability level.

Discussion

The results of this study demonstrate a statistically significant negative association between A_{540} (LAC content) and HCN-p (dhurrin content) in sorghum leaves. The magnitude of the r values is such, however, that a close relationship between these variables is not indicated. Therefore, it is not likely that either trait could be used as a reliable indicator of the other in selection or breeding programs, and livestock consuming sorghum forage that is high in LAC should not automatically be assumed to be free of the danger of cyanide poisoning.

The observed negative association might possibly be the result of linkage between the major gene for LAC content and one or more genes influencing HCN-p level. It is also reasonable to suggest that the negative association might be the result of metabolic relationships between LAC and dhurrin, without conventional linkage between the genes that are involved. The formation of both LAC and dhurrin depends in part on aromatic biosynthesis. Aromatic intermediates that are used in dhurrin formation cannot be available for LAC synthesis, and vice versa. Of course, aromatic intermediates also are involved in many other metabolic pathways in plants, and such involvement would be expected to weaken the relationship between LAC and dhurrin contents. Therefore, a significant but not very strong relationship between contents of these two constituents is not surprising.

The rationale just described cannot be applied to the negative association reported by Ross and Jones (1983) for *L. corniculatus.* The cyanogenic compounds of *L. corniculatus* are linamarin and lotaustralin (Seigler 1976), neither of which has an aromatic moiety. Therefore, the negative association between contents of tannin and cyanide in *L. corniculatus* probably does not involve competition for intermediates or products of the aromatic biosynthetic pathway. Ross and Jones (1983) used the procedure of Joseph and Gaur (1971) for cyanide determination. This procedure depends upon endogenous enzymes for the release of cyanide from cyanogenic glucosides. Goldstein and Spencer (1985) have shown clearly that tannins inhibit the β-glucosidase-mediated hydrolysis of cyanogenic glucosides *in vitro.* Therefore, it seems possible that the negative association observed by Ross and Jones may have resulted from differing levels of tannin, and thus differing degrees of inhibition of the hydrolysis of linamarin and lotaustralin during the cyanide assay, rather than from an actual inverse relationship between contents of tannin and the cyanogenic glucosides in plant leaves. Assays of cyanogenic compounds in tannin-containing leaves should be designed in such a way that endogenous enzymes are not involved in the assay.

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