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Inheritance of Leucoanthocyanidin Content in Sorghum Leaves[1]

F. A. Haskins and H. J. Gorz[2]

ABSTRACT

Leucoanthocyanidins (LAC) are structurally related to condensed tannins, a class of compounds having significant effects on the nutritional value of sorghum [*Sorghum bicolor* (L.) Moench] grain. Little is known of LAC inheritance in sorghum. Mature leaves of 'Colman' and 'White Collier' forage sorghum are high and low, respectively, in LAC content. The objective of the present study was to determine the inheritance of LAC content in reciprocal crosses between these two cultivars. Leaves of field-grown plants were assayed for LAC by a procedure that involved treating methanolic leaf extracts with acid at 50°C to convert the colorless LAC to an anthocyanidin having an absorbance maximum near 540 nm. Parental, F_1, and F_2 plants were grown and assayed in 1983. In 1984, F_3 progenies from selected F_2 plants representing various LAC levels were grown and assayed along with additional F_2 and parental plants. Results from both years supported the conclusion that a single allelic pair with incomplete dominance was primarily responsible for the difference in LAC content between Colman and White Collier plants. Other genes with minor effects may also be involved, but maternal or cytoplasmic effects were not important in determining LAC level. The LAC of Colman was not identified, but based on the work of other researchers with other sorghums, it is probably apiforol.

Additional index words: **Apiforol, Flavonoids, Genetics, *Sorghum bicolor* (L.) Moench, Tannins.**

THE terms, leucoanthocyanidin (LAC), proanthocyanidin (PAC), and tannin, are not always clearly defined or distinguished in the literature. In their recent report on the occurrence of apiforol in sorghum [*Sorghum bicolor* (L.) Moench] leaves, Watterson and Butler (11) used LAC to include monomers of flavanols such as flavan-3,4-diols and flavan-4-ols, and PAC (or condensed tannin) to include oligomers of flavan-3-ols. This terminology is used in the present report. Treatment with acid can convert both LAC and PAC to colored anthocyanidins, the former by loss of water from the monomeric molecules and the latter by depolymerization.

Watterson and Butler (11) investigated leaf tissue and grain of 43 grain sorghum lines for the presence of LAC and PAC. Twenty-seven of the lines had grain PAC, but none contained PAC in the leaves. Twelve lines had LAC in their leaves; all of these plus three additional lines had grain LAC. Most of the lines with leaf LAC had grain PAC, but this was not true of all lines.

The PAC of sorghum grain is believed to be responsible for both desirable (e.g., bird resistance) and undesirable (e.g., decreased nutritional value) effects (3, 11). The function of LAC within the sorghum leaf and the influence of LAC on utilization of the forage have not been firmly established.

Woodruff et al. (12) studied 13 crosses involving 22 different lines in an investigation of the inheritance of tannin content in sorghum grain. They concluded that tannin quantity was a highly heritable trait and that high content was dominant to low. However, they reported that simple one- or two-gene models were inadequate to explain all of their data. The assay procedure used by Woodruff et al. provided for correction of PAC levels for interference by LAC. The work of Sieglinger (7) and Stephens (8) led to the conclusion that the presence in the sorghum kernel of pigmented testa, now known to be associated with high tannin content, is controlled by the complementary gene pairs, *B/b* and *B2/b2*. None of these reports on grain tannins was concerned with the inheritance of tannins or related compounds in sorghum leaves.

Paroda et al. (5) studied the inheritance of forage tannin content in a 6 × 6 diallel involving six *Eu-sorghum* species, and they concluded that level in the forage was highly heritable with low content dominant to high. Boora and Lodhi (1) used 17 lines and five testers in a study of toxic constituents in forage sorghum; they found a preponderance of nonadditive genetic variance for tannin content. The vanillin-HCl assay procedure described by Burns (2) was used in both of the investigations just cited. This method does not distinguish clearly between PAC and LAC. However, if the findings of Watterson and Butler (11) apply to sorghums in general, it may be assumed that LAC was the substance assayed as tannin in these two studies.

Reyes-Discua (6) also reported that forage tannin content was genetically controlled. This conclusion was based on two sets of diallel crosses involving sorghum lines representing various levels of leaf tannin. No estimate was made of the number of genes involved. Reyes-Discua used a modified vanillin-HCl procedure (4), which differed somewhat from the procedure of Burns (2), but neither of these procedures distinguished between LAC and PAC.

Walton (9) assayed the same plant samples used by Reyes-Discua, applying a procedure that detected LAC but not PAC. His conclusions regarding inheritance were similar to those of Reyes-Discua (6).

The LAC assay described by Walton et al. (10) revealed differences of 20-fold or more between leaves of 'White Collier' (low in LAC) and 'Colman' (high in LAC) forage sorghum. We reasoned that a genetic study involving only these two cultivars might provide relatively straightforward information regarding the inheritance of LAC content in sorghum leaves. This report presents the results of such a study.

MATERIALS AND METHODS

Production and Sampling of Plants

Plants of Colman and White Collier were grown at the Agronomy Farm, Lincoln, NE, during the summer of 1981, and reciprocal crosses between the cultivars were made by hand emasculation and pollination. Field-grown F_1 plants were self-pollinated in 1982 to produce F_2 seed. In 1983, both parental cultivars, reciprocal F_1's, and F_2's, were started in the greenhouse and were transplanted to the Agronomy

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Farm. Plants were spaced 0.61 m apart in rows that were 0.76 m apart. In August 1983, as the plants reached full head-emergence, the blade of the flag leaf from the main stalk of each plant was sampled for assay as previously described (10). These leaf blades were wiped free of dust, midribs were removed and discarded, and the remaining tissue was dried overnight at 75°C. Dried tissue was ground through a small Wiley[3] mill equipped with a 1-mm screen, and ground samples were held in a freezer until they were extracted for assay. In 1983, 20 plants of each parent, 38 F_1's, and 179 F_2's were assayed, and self-pollinated seed was harvested from each of these plants.

Seed from selected plants from the 1983 nursery was planted in the greenhouse in the spring of 1984, and 660 plants were transplanted to the University of Nebraska Mead Field Laboratory on 21 and 24 May. Plant and row spacings were the same as in the 1983 nursery. The 1984 nursery was arranged in six replications, each of which consisted of 11, 10-plant rows. The following 11 entries were randomly assigned to these rows: White Collier, Colman, F_2 of Colman × White Collier, F_2 of White Collier × Colman, and F_3 families from seven classes of F_2 plants, each representing a different LAC level. Each of these seven classes included six different F_2 plants; thus, 42 F_2 plants were advanced to the F_3 generation. In all, 658 plants survived in the 1984 nursery. These plants were sampled on 25 and 26 July 1984. At sampling, heads were at least partially emerged from about 90% of the plants. Except that plants generally were somewhat less mature at sampling in 1984 than in 1983, sampling procedure and sample treatment were similar in both years.

Extraction and Assay

The procedures described by Walton et al. (10) were followed for tissue extraction and assay. Samples (100 or 200 mg) of dried and ground tissue were extracted overnight at room temperature with methanol (CH_4O) (20 mg of tissue mL^{-1}). A 0.25 mL portion of extract was transferred to a Bausch and Lomb[3] colorimeter tube, and 1.0 mL of chloroform ($CHCl_3$) was added. To the resulting solution, 1.75 mL of a mixture of methanol, chloroform, and 6 *M* HCl (19:4:12, v/v/v) was added, and the mixture was shaken vigorously and immediately placed in a 50°C water bath to allow color development and phase separation. At precisely 20 min, the tube was removed from the bath and swirled gently to mix the methanol-HCl phase (which was sometimes nonhomogeneous because of refluxing), and the absorbance of this phase at 540 nm was immediately read with a Spectronic 20[3] colorimeter. All results were expressed as A_{540} readings made under these conditions.

RESULTS AND DISCUSSION

Mean A_{540} values for Colman were much higher than those for White Collier in both 1983 and 1984 (Table 1). The difference between the cultivars was less pronounced in 1984 than in 1983, probably reflecting differences in the environment under which the plants were grown as well as variation in plant maturity at sampling. Walton (9) observed that A_{540} values for leaf samples increased slightly between the boot stage and anthesis and then decreased during grain development, and Watterson and Butler (11) reported that apiforol content of fully expanded leaves decreased as the leaves aged.

Table 1. Values of A_{540} obtained in 1983 and 1984 for sorghum parents [Colman (C) and White Collier (WC)], reciprocal F_1's and F_2's. F_2 plants classified as leucoanthocyanidin positive (LAC+) had A_{540} readings of no less than 0.024 in 1983 and 0.061 in 1984.

Year	Entry	No. of plants	A_{540}
			Mean ± SE
1983	Parents		
	C (LAC+)	20	0.810 ± 0.052
	WC (LAC−)	20	0.010 ± 0.001
	F_1		
	C × WC	18	0.255 ± 0.016
	WC × C	20	0.282 ± 0.020
	Combined F_1	38	0.269 ± 0.013
	F_2†		
	LAC+	132	0.377 ± 0.022
	LAC−	47	0.011 ± 0.001
1984	Parents		
	C	60	0.613 ± 0.037
	WC	60	0.023 ± 0.001
	F_2†		
	LAC+	87	0.268 ± 0.019
	LAC−	32	0.032 ± 0.002

† Chi-square tests for goodness-of-fit to a 3 LAC+:1 LAC− ratio in F_2 were: $\chi^2 = 0.15, P = 0.70$ in 1983; $\chi^2 = 0.23, P = 0.63$ in 1984.

The reciprocal F_1's were intermediate in A_{540} level and did not differ significantly from each other (Table 1). This lack of difference suggested that maternal or cytoplasmic effects were not of major importance in determining LAC content in these crosses. Therefore, data from the reciprocal crosses were pooled to provide the best estimate of A_{540} for the heterozygous F_1's, and data from the reciprocal F_2's also were pooled.

Cursory examination of all 1983 data (Table 1) suggested that LAC level was controlled by a single pair of alleles with Colman and White Collier being homozygous for high and low levels, respectively. The mean A_{540} of the heterozygous F_1 plants (0.269) ± 3 SD units was used to classify F_2 plants into high ($A_{540} > 0.514$), intermediate (A_{540} from 0.024 to 0.514), and low ($A_{540} < 0.024$) groups. However, the resulting F_2 segregation, 30 high:102 intermediate:47 low, provided a poor fit to a 1:2:1 ratio ($\chi^2 = 6.72$, $P = 0.03$). When intermediate and high classes were combined into a single LAC+ class, a segregation of 132:47 and a close fit to the monogenic 3:1 ratio resulted (Table 1). The poor fit to a 1:2:1 ratio probably was caused by incorrect classification of some of the intermediate plants as heterozygous rather than homozygous for high content.

No F_1 plants were grown in 1984, thus, it was not possible to use F_1 values to establish limits between LAC+ and LAC- plants. For both F_2 and F_3 data in 1984, we based this classification on the A_{540} values of the six F_3 families (60 plants) derived from six F_2 plants with A_{540} readings < 0.050 (the actual range was 0.002 to 0.018). Each of these six F_3 families bred true for low LAC content. Their mean A_{540} was 0.032, and this value + 3 SD units was 0.060. In 1984, plants with A_{540} values from 0 to 0.060 were classified as LAC-, and those with values greater than 0.060 as LAC+. No attempt was made to separate LAC+ plants into intermediate and high groups in 1984.

The F_2 segregation in 1984 was similar to that observed in 1983 (Table 1). A close fit to a 3 LAC+:1LAC- ratio was indicated. Pooling of the F_2 data for both

[3] Names of products are included for the benefit of the reader and do not imply endorsement or preferential treatment by the USDA or the Univ. of Nebraska.

Table 2. Classification of F_3 sorghum plants as leucoanthocyanidin positive (LAC +) or negative (LAC −), and A_{540} means and standard errors from segregating (S) and nonsegregating (NS) F_3 families derived from F_2 plants with known A_{540} values. Plants classified as LAC + had A_{540} readings of no less than 0.061. All plants were grown in 1984.

F_2 plants	F_3 families		F_3 plants				
A_{540} range	Type	No.	No.	LAC class	A_{540}	χ^2†	P
>0.549	NS	6	60	+	0.482 ± 0.036		
0.450–0.549	NS	4	40	+	0.486 ± 0.035		
	S	2	12	+	0.203 ± 0.026	2.40	0.12
			8	−	0.034 ± 0.003		
0.350–0.449	NS	2	20	+	0.436 ± 0.032		
	S	4	29	+	0.357 ± 0.037	0.01	0.93
			10	−	0.030 ± 0.003		
0.250–0.349	S	6	43	+	0.263 ± 0.024	0.36	0.55
			17	−	0.032 ± 0.003		
0.150–0.249	S	6	44	+	0.284 ± 0.030	0.09	0.77
			16	−	0.032 ± 0.002		
0.050–0.149	S	6	46	+	0.188 ± 0.019	0.09	0.77
			14	−	0.036 ± 0.002		
<0.050	NS	6	60	−	0.032 ± 0.001		
Combined segregating F_3 families		24	174	+	0.260 ± 0.013	0.62	0.43
			65	−	0.033 ± 0.001		

† Chi-square tests are for goodness-of-fit to a 3 LAC+:1 LAC− ratio in segregating F_3 families.

years resulted in totals of 219 LAC+ and 79 LAC−, which also provided a good fit to a 3:1 ratio (χ^2 = 0.36, P = 0.55)

All F_3 plants from F_2 individuals with A_{540} values >0.549 were LAC+; those from F_2 plants with A_{540} values <0.050 were LAC- (Table 2). Four of the F_3 families from F_2 plants with A_{540} values between 0.450 and 0.549 were all LAC+, and the other two F_3 families from this group segregated for LAC+ and LAC−. Among F_3 families from F_2 plants having A_{540} values between 0.350 and 0.449, four segregated for LAC content and two were all LAC+. The presence of both segregating and nonsegregating F_3 families from the latter two classes of F_2 plants emphasized our inability to distinguish between homozygous and heterozygous F_2 plants in these A_{540} ranges. All F_3 families from F_2 plants in the three ranges, 0.050 to 0.149, 0.150 to 0.249, and 0.250 to 0.349, segregated for LAC content. As shown in Table 2, each group of segregating families provided a satisfactory fit to the monogenic 3:1 ratio, and the combined data from all segregating F_3 families also fit this ratio.

We concluded from the foregoing observations that a single allelic pair was primarily responsible for the difference in LAC content of Colman and White Collier leaves. The background in which the alleles functioned doubtless had some effect on A_{540} level, however, as shown by the data in Tables 1 and 2. The mean value for the nonsegregating low F_3 families (0.032) was slightly larger than the White Collier mean (0.023), and the nonsegregating high F_3 families (means from 0.436 to 0.486) were somewhat lower than the Colman mean (0.613).

We have not isolated the LAC responsible for the development of red color when methanol extracts of Colman leaves were acidified and heated at 50°C. However, we observed that, when the LAC was heated in 2 M HCl in a boiling water bath for 15 min, the red color disappeared and the solution had an absorbance maximum near 480 nm. This behavior was similar to that described by Watterson and Butler (11) for apiforol. It is reasonable to suggest that the LAC, apiforol, occurs not only in the lines examined by Watterson and Butler but also in Colman and other lines that yield high A_{540} values in the chloroform-HCl assay (9, 10).

Each of the plants included in this study was subjected to a rapid spot test at the time samples were taken for the A_{540} assay. A piece of flag-leaf-blade tissue about 15 mm^2 in area was crushed on Whatman no. 1 filter paper and allowed to dry. A drop of freshly prepared solution consisting of 10 mL of 95% ethanol, 5 mL of concentrated HCl, and 1 g of vanillin was added to each spot of crushed tissue. Spots were scored as + (red color) or − (no red color) after the sheets were completely dry. Results generally agreed well with the A_{540} assay, but a small number of plants that were scored as − in the spot test had A_{540} values slightly above the limit designated for −. We found that results of the spot test were much more definitive when vanillin was included in the test solution, although vanillin was not used in the A_{540} assay.

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