University of Nebraska - Lincoln Digital Commons@University of Nebraska - Lincoln

Agronomy & Horticulture -- Faculty Publications

Agronomy and Horticulture Department

1-1-1960

Genetic Blocks in the Synthesis of Coumarin in Melilotus alba

Herman J. Gorz United States Department of Agriculture

Francis A. Haskins University of Nebraska - Lincoln, fhaskins@neb.rr.com

Follow this and additional works at: http://digitalcommons.unl.edu/agronomyfacpub



Part of the Plant Sciences Commons

Gorz, Herman J. and Haskins, Francis A., "Genetic Blocks in the Synthesis of Coumarin in Melilotus alba" (1960). Agronomy & Horticulture -- Faculty Publications. Paper 276.

http://digitalcommons.unl.edu/agronomyfacpub/276

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska -Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

GENETIC BLOCKS IN THE SYNTHESIS OF COUMARIN

In Melilotus alba

H. J. Gorz and F. A. Haskins*

OUMARIN synthesis in sweetclover (Melilotus alba) appears to ✓ be primarily controlled by two independent pairs of genes designated as Cu/cu and $B/b^{1,3}$. When homozygous, the cu gene produces a marked reduction in the level of total coumarin while the b gene prevents the formation or accumulation of appreciable quantities of free coumarin in the plant. Plants of the Cu B phenotype contain both free and bound coumarin, plants of the Cu b phenotype contain bound coumarin but are very low in free coumarin, and plants of the cu B and cu b phenotypes are low in both forms of coumarin. It is reasonable to suppose that the two gene pairs affect, either directly or indirectly, certain steps in the biosynthesis of the coumarin molecule. In the terminology of the chemical geneticist, it might be said that the cu and b genes, when homozygous, block specific reactions concerned with coumarin synthesis. Such biosynthetic blocks are well known, particularly in Neurospora crassa and other microorganisms⁵. It is the purpose of this paper to present the results of experiments in which improved assay methods have been used to measure the influence of the two genes, cu and b, upon the level and form, respectively, of coumarin in sweetclover leaf tissue.

Materials and Methods

Twenty-five sweetclover plants of each of the four homozygous genotypes, CuCuBB, CuCubb, cucuBB, and cucubb, were used in this study. The plants of

known genotype were chosen from several F₈ lines that had been derived from a single, doubly heterozygous F₆ plant. In the original cross from which this F_6 plant was derived, a cucuBB plant was used as the female parent and a CuCubb plant as the male parent. (Acknowledgment is made to Dr. W. K. Smith at the University of Wisconsin for the parental material.) Ten alfalfa plants also were assayed to provide an indication of the magnitude of fluorescence not ascribable to coumarin. Assays for free and bound coumarin were made with the use of the Beckman model DU spectrophotometer equipped with the fluorescence attachment. Young leaf tissue was used, and the procedure described by Haskins and Gorz³ was followed.

Results and Discussion

Average levels of free, bound, and total coumarin, with standard errors, for each of the four homozygous genotypes of sweetclover and for alfalfa are shown in Table I. In Table II, levels of free and bound coumarin are expressed as percentages of the total coumarin content.

TABLE I. Average coumarin content of sweetclover leaves representing plants of four genotypes and of alfalfa leaves

	Coumarin equivalence (dry weight basis)			
	Free	Bound	Total	
Genotype	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	
	%	%	%	
cucubb	0.02002	0.20022	0.22022	
cucuBB	0.08013	0.16030	0.24037	
CuCubb	0.02001	5.13268	5.15268	
CuCuBB	1.09102	3.05315	4.14317	
Alfalfa	0.01001	0.01001	0.02001	

*Geneticist, Crops Research Division, A.R.S., U.S.D.A., and Agronomist, Nebraska Agr. Exp. Sta., Lincoln, Nebraska. Joint contribution of the Crops Research Division, A.R.S., U.S.D.A., and the Nebraska Agr. Exp. Station. Published with the approval of the Director as paper No. 875, Journal Series, Nebraska Agricultural Experiment Station.

Gorz and Haskins: Genetic Blocks in Synthesis of Coumarin

It is apparent from the data that in plants which are homozygous with respect to cu, the synthesis of coumarin is not completely blocked. Although the average level of total coumarin in cucu plants is appreciable and easily detectable, amounting to approximately 0.2 percent of the dry weight, the level is only about 1/20 of that found in CuCu plants. The degree of fluorescence noted in assays of cucu plants, however, is 11.5 times that observed for alfalfa, and it must be concluded that the cu-effected block in coumarin synthesis is partial rather than complete.

The b gene, on the other hand, appears to be highly effective in blocking the formation of free coumarin. Thus, in plants of the CuCubb genotype, less than one percent of the total coumarin content is in the form of free coumarin. In cucubb plants the proportion of free coumarin appears to be somewhat larger, amounting to about nine percent. However, for both of these genotypes the free coumarin levels listed in Table I are almost as low as the value recorded for alfalfa. It is probable, therefore, that little if any of the "free coumarin" fluorescence noted in assays involving these genotypes is actually due to coumarin. This very small amount of "free coumarin' accounts for a greater percentage of the total coumarin content in plants of the cucubb genotype than in CuCubb plants because of the low total coumarin content of the former plants. In view of these considerations, it may be assumed that the values of 0.4 percent free coumarin and 99.6 percent bound coumarin listed in Table II for the CuCubb genotype probably represent the upper limit for the free form and the lower limit for the bound form, respectively, in plants which are homozygous for b. Thus, a virtually complete blocking action is indicated for the b gene.

Information thus far available indicates that bound coumarin, free coumarin, and the cu and b genes are related as follows:



As indicated in this proposed pathway, bound coumarin appears to serve as a precursor of free coumarin in the plant, the cu gene partially blocks the conversion of unspecified precursors to bound coumarin, and the b gene prevents the conversion of bound coumarin to the free form. Further work on this biosynthetic pathway is planned.

The results reported in this paper have an important bearing on sweetclover breeding. One of the primary objectives in sweetclover breeding programs is the development of strains that are free of coumarin, since this chemical imparts a bitter taste to the forage and also gives rise to the toxic material, dicoumarol, which occurs in spoiled sweetclover hay and silage. Dicoumarol is the caus-

ative agent associated with the so-called 'sweetclover disease" in livestock4. Breeding for low coumarin level requires the analysis of many thousands of plants for coumarin content. Of necessity this large-scale analysis must be done qualitatively. Qualitative fluorometric procedures2 now available for use by the plant breeder permit the detection of very low levels of coumarin. The plant breeder should be aware that the cu-effected block in coumarin synthesis is partial in nature, and that some coumarin should, therefore, be expected in plants having the cucu genotype. An application of this knowledge may save much valuable germplasm that might otherwise be discarded as unsuitable.

It is also important to know the level of coumarin in plants of each of the various genotypes when utilizing the coumarin-conditioning alleles as genetic markers, or in studies of the physiology of coumarin synthesis in sweetclover.

Summary

The influence of the two genes, cu and b, upon the level and form of coumarin in sweetclover leaf tissue was determined by assaying 25 sweetclover plants of each of the four homozygous genotypes, CuCuBB, CuCubb, cucuBB, and cucubb. An assay of alfalfa leaves provided an indication of the magnitude of fluorescence not ascribable to coumarin. Approximately 0.2 percent total coumarin (dry weight basis) was found in plants homozygous for cu, which is 11.5 times the amount found in alfalfa, but only about 1/20 of the level found in CuCu plants. Thus, the cu-effected block in coumarin synthesis is partial rather than complete. However, the action of the b gene in blocking the formation of free commarin is virtually complete, as shown by the extremely low levels of free coumarin in bb plants. The probable relationship of bound coumarin, free coumarin and the cu and b genes is shown. Important implications in sweetclover breeding, and in other studies are indicated.

Literature Cited

1. Goplen, B. P., J. E. R. Greenshields and H. Baenziger. The inheritance of commarin in sweetclover. *Canadian Jour. Bot.* 35:583-593. 1957.

TABLE II. Levels of free and bound coumarin in four genotypes of sweetclover, expressed as percentages of total coumarin content

Genotype		Percentage of total content occurring as	
	Total coumarin	Free	Bound
	% of dry wt.		
cucubb	0.22	9.1	90.9
cucuBB	0.24	33.3	66.7
CuCubb	5.15	0.4	99.6
CuCuBB	4.14	26.3	73.7

The Journal of Heredity

76

- 2. Gorz, H. J. and F. A. Haskins. Rapid tests for free and bound coumarin in sweet-clover. Agron. Jour. 50:211-214. 1958.
 3. Haskins, F. A. and H. J. Gorz. Fluorometric assay of free and bound coumarin in sweetclover. Agron. Jour. 49:493-497, 1957.
- 4. LINK, K. P. The anticoagulant from spoiled sweetclover hay. *The Harvey Lecture Series* 39:162-216. 1943-1944.
 5. Wagner, R. P. and H. K. MITCHELL. Genetics and Metabolism. John Wiley and Sons. New York. 1955.