

# Expression of the Gene d1 in the Scutellum of Maize

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## Recommended Citation

Pelton, Jeanette S. (1954) "Expression of the Gene d1 in the Scutellum of Maize," *Butler University Botanical Studies*: Vol. 11, Article 21.

Available at: <http://digitalcommons.butler.edu/botanical/vol11/iss1/21>

**Butler University**  
**Botanical Studies**  
(1929-1964)

*Edited by*

**J. E. Potzger**

The *Butler University Botanical Studies* journal was published by the Botany Department of Butler University, Indianapolis, Indiana, from 1929 to 1964. The scientific journal featured original papers primarily on plant ecology, taxonomy, and microbiology. The papers contain valuable historical studies, especially floristic surveys that document Indiana's vegetation in past decades. Authors were Butler faculty, current and former master's degree students and undergraduates, and other Indiana botanists. The journal was started by Stanley Cain, noted conservation biologist, and edited through most of its years of production by Ray C. Friesner, Butler's first botanist and founder of the department in 1919. The journal was distributed to learned societies and libraries through exchange.

During the years of the journal's publication, the Butler University Botany Department had an active program of research and student training. 201 bachelor's degrees and 75 master's degrees in Botany were conferred during this period. Thirty-five of these graduates went on to earn doctorates at other institutions.

The Botany Department attracted many notable faculty members and students. Distinguished faculty, in addition to Cain and Friesner, included John E. Potzger, a forest ecologist and palynologist, Willard Nelson Clute, co-founder of the American Fern Society, Marion T. Hall, former director of the Morton Arboretum, C. Mervin Palmer, Rex Webster, and John Pelton. Some of the former undergraduate and master's students who made active contributions to the fields of botany and ecology include Dwight W. Billings, Fay Kenoyer Daily, William A. Daily, Rexford Daudenmire, Francis Hueber, Frank McCormick, Scott McCoy, Robert Petty, Potzger, Helene Starcs, and Theodore Sperry. Cain, Daudenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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# EXPRESSION OF THE GENE $d_1$ IN THE SCUTELLUM OF MAIZE

By JEANETTE S. PELTON

There have been many descriptions of the morphological expression of mutant genes in the mature plant body, but relatively little has been done on gene expression in early stages of ontogeny in higher plants. The fact that many genes have marked expression in older plants raises the question of the extent to which they are manifested at earlier stages of development. In the present study an embryonic structure of maize, the scutellum, is investigated for evidence of expression of a simple Mendelian recessive, dwarf-one ( $d_1$ ). Four different inbreds were used because it was anticipated that quantitative expression of the gene would be modified by association with different genotypes. The scutellum was chosen as the specific subject of study since preliminary investigations indicated that scutellum shape varied from one inbred to another. It seemed possible, then, that a single gene difference might also show some expression in this organ.

Presence of the gene  $d_1$  is clearly apparent at maturity; plants homozygous recessive for this gene are considerably lower in stature and have shorter and wider leaves than do their normal sibs (Abbe, L. 1936). The gene  $d_1$  is located on the third chromosome and segregates in a three to one ratio (Emerson, Beadle, and Fraser 1935).

## MATERIALS AND METHODS

### Source of Materials:

The maize kernels used in this study were the product of a backcrossing program which has extended through a number of generations. The original material carrying the gene  $d_1$  was backcrossed to four University of Minnesota Agriculture Experiment Station inbreds; A21, A25, A172, and A188. In summary, the breeding program that produced the segregating cultures is as follows:

1940	$d_1d_1$ ♂	X ♀	Station	Inbred	$P_1$
1941	$D_1d_1$ ♂	X ♀	"	"	$F_1$
1942	$D_1d_1$ ♂	X ♀	"	"	$F_2$ ( $D_1D_1$ X Station Inbred discarded)

1944  $D_1d_1 \delta \times \text{♀}$  " "  $F_8$  " " " " "  
 1945  $F_2 \boxtimes$  (non-segregating progeny discarded)  $F_3 \boxtimes$   
 1948  $(F_2 \boxtimes) \boxtimes$  (non-segregating progeny discarded)  $F_3 \boxtimes \boxtimes$

This program was essentially followed for all the inbred cultures, except that in A25 four generations of backcrossing rather than the usual three years preceded the two years of selfing.

Although normal sibs in the material segregating for  $d_1$  differ slightly from the Station inbreds because of the introduction of the gene  $d_1$  from a foreign background some three or four generations earlier, the Experiment Station's original designations will be used for the inbred cultures studied.

#### Characteristics of the Four Inbred Lines:

Field observations of the four Station inbreds for several generations have shown that the inbreds differ in certain seedling and mature plant characteristics. Major differences observed in characteristics of mature plants are height, leaf shape, and leaf color. A brief description of these differences is as follows: In height A25 is the tallest, A172 second tallest, A188 third, and A21 the shortest; leaf shape differs with A188 having the widest leaves, A21, A25, and A172 having narrow leaves, A172 being the narrowest; leaf color ranges from dark green in A188 to the lightest green of the group in A21. A25 differs from the other inbreds in having rather poor germination qualities as compared to the excellent germination qualities for the other three. Time of germination marks a definite distinction between the four inbreds; A21 germinates first, A172 second, A188 third, and A25 last. In addition to these differences the writer's measurements of the scutella from mature kernels show that the Station inbreds also differ in size and form of the scutellum in each line. The mathematical means for length, width, and depth of excised scutella are given in Table I.

Thus, mature plant characteristics as well as germination traits indicate genetic differences in the four Station inbreds. This genetic difference is also expressed in the scutellum with notable differences in form: A21 has the shortest scutellum, A188 the widest scutellum, and that of A172 has the greatest depth. Since such marked differences were correlated with major genetical differences, it seemed not unlikely that a single gene difference might also be expressed in the scutellum.

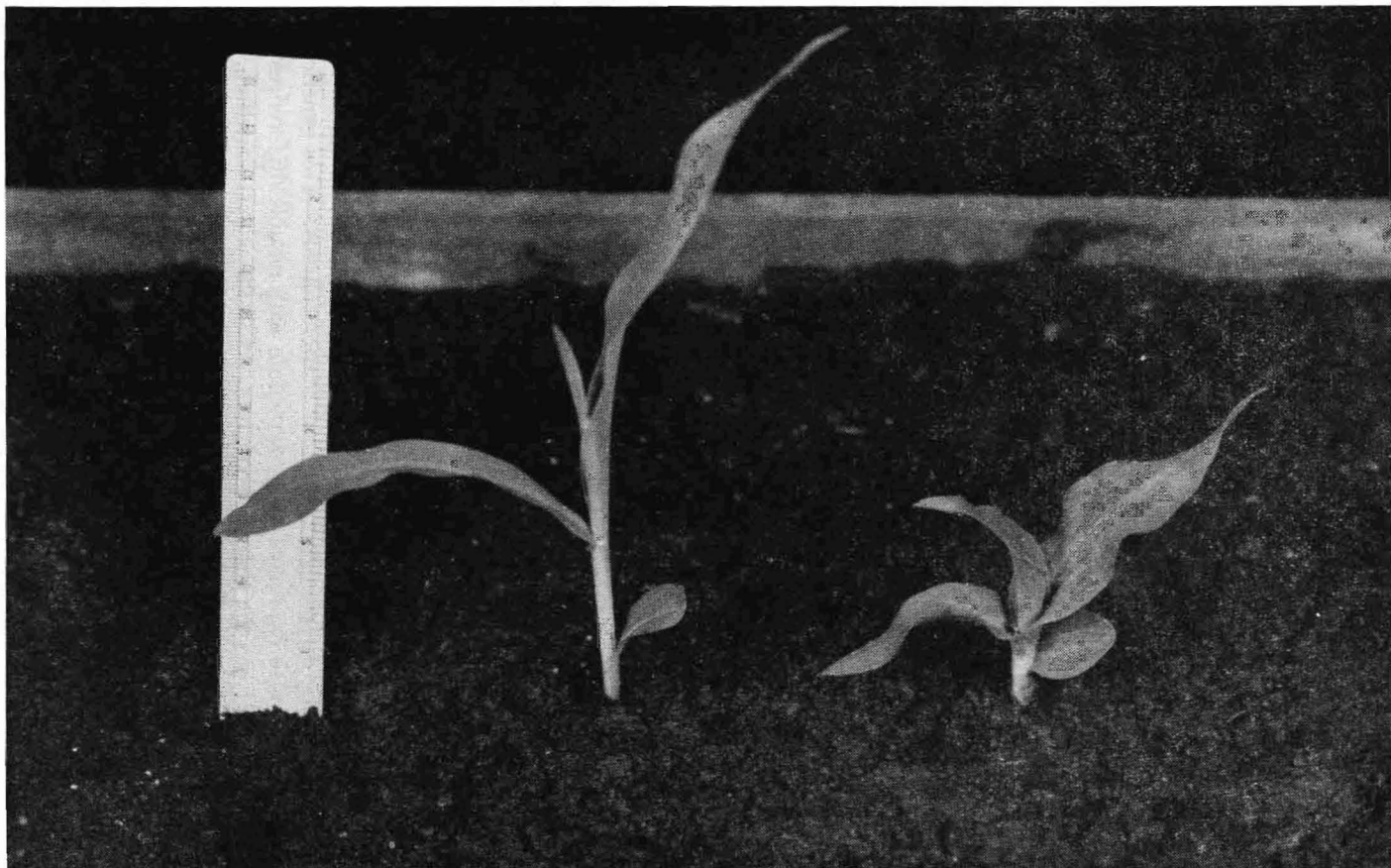


Fig. 1. Ten day old seedlings of a normal sib and the homozygous recessive dwarf-one of Inbred culture A21.

## Experimental Procedure:

Shelled kernels from a single ear of each of the four inbred segregating cultures were used and included about 200 to 250 kernels of each inbred culture. Throughout the experiments environmental conditions were kept as nearly uniform as possible. Simultaneous soaking of ten kernels of each inbred culture in distilled water was the first step of each experiment. Forty-eight hours later maximum length and width of scutellum and kernel were measured to 0.2 mm using 13.8X magnification. A critical feature in the experimental procedure was the determination of the genetical identity of each scutellum. This could be assured only by growing the plants until the characteristic difference in form of the seedling leaves permitted the identification of the homozygous recessives. Figure 1 illustrates this phenotypic difference in the ten day old seedlings. Therefore, each individual measured scutellum received a number which was given phenotypic identification at the seedling stage. Thirteen such experiments were completed between April 5, 1949 and June 5, 1949.

## EXPERIMENTAL RESULTS

Average length of the scutellum in the dwarf embryos as compared with that of its normal sibs does not differ statistically in any of the four inbred cultures. Statistical treatment included calculation of mathematical mean, standard deviation, and the probability of significant differences between mathematical means. The latter calculation was made by using the following formula:

$$k = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{N_1 - 1} + \frac{s_2^2}{N_2 - 1}}}$$

$\bar{X}_1$  and  $\bar{X}_2$  refer to the means of the normal and dwarf measurements.

$s_1^2$  and  $s_2^2$  refer to the square of the standard deviations of the two populations.

$N_1$  and  $N_2$  refer to the numbers of individuals in the two populations.

$k$  is the ratio between observed difference and its standard error and is used with a table of normal curve functions (Treloar 1939, pp. 240-243).

Only one case of a statistically significant difference in dwarf and normal means is found in scutellar width: That difference being the larger mathematical mean of the normal in the A21 culture, with a probability of 0.02%. These statistical results are summarized in Table II.

Analysis of length and width of the kernel dimensions using the same statistical treatment as in scutellar analysis did not produce any statistically significant differences between dwarf and normal mathematical means. Statistical results of kernel measurements are summarized in Table III.

## DISCUSSION

Results of the present study indicate that although major genetical differences between inbred lines of maize may be reflected in scutellar size and form, similar differences were not correlated with  $d_1$  in the homozygous recessive state. While the phenotype of this recessive gene is noticeably different from its normal sibs as early in development as the very young seedling, a corresponding expression in the scutellum is not found. Thus, the numerous genes that control size and shape of scutellum are found to differ in the Station inbreds, but an introduction of a single gene difference does not seem to alter any of these gene complexes at this early stage.

In view of this lack of expression of the gene  $d_1$  in kernel as well as scutellar form, a consideration of the role of environmental influences seems in order. Among external factors that might alter kernel size and shape are: Nutrition, pressure of the surrounding kernels, and moisture and temperature during development of the kernels. An attempt was made to equalize such positional effects as pressure and distance from the vascular tissue supplying nutrients and water, and the resulting competition among the kernels, by using all the kernels from a single ear for each of the inbred lines. Moisture and temperature during development of the kernels are variables common to all four cultures because they were grown during the same season and in the same uniform field plots. Therefore, while external factors can be important in affecting kernel form, the in-



fluence of these factors would seem sufficiently uniform to the individuals in the experiment to rule out environmental conditions as a major factor in modifying genetic expression of a gene such as  $d_1$ , that so completely modifies many characteristics of maize.

Among the factors which might affect scutellar form the relationship between kernel form and scutellar dimensions should not be overlooked. A high correlation between scutellar and kernel dimensions might indicate that the caryopsis wall can have some influence on scutellar size. Scatter diagrams of kernel and scutellar length and width, however, indicate only weak correlations or none at all. Consequently, these data do not support the theory that surrounding tissues have some restricting action on embryo dimensions in this experiment.

The genetic composition of the scutellum in the material analyzed in the present study can be divided into three classes: The homozygous recessive,  $d_1d_1$ , the homozygous dominant,  $D_1D_1$ , and the heterozygous,  $D_1d_1$ . This is in marked contrast to the genetic uniformity of the caryopsis wall which is consistently  $D_1d_1$ . Interestingly enough, even though the seedlings which developed from the measured embryos could be classified either as phenotypically dwarf or normal, a corresponding phenotypic expression of  $d_1$  in the scutellum is not found. Against three of the inbred backgrounds (A25, A188, and A172) the mathematical means of the scutella of the dwarf embryos did not differ statistically either in length or width. The only statistically significant difference is in the A21 strain in which the mathematical mean of the scutellum of the normal is larger in width than that of the dwarf. In this case the actual difference between dwarf and normal means is only 0.4 mm, which is a rather slight difference since measurements were made to only 0.2 mm. Thus, it would seem that the  $d_1$  gene has little or no influence on the genes controlling embryonic size in either the dwarf or normal embryos. A possible explanation is that the threshold of action of  $d_1$  may come after the completion of embryonic growth.

## SUMMARY

The purpose of the investigation described above was to determine whether the gene  $d_1$  is expressed in the mature embryo of maize. Four inbred lines, each segregating for the gene, were used

in the experiment. The method of study involved measurements of maximum length and maximum width of scutellum and kernel. Identification of the gene was possible through the use of a numbering system assuring the correct phenotype at the seedling stage. Statistical analysis of the data shows only one case of a statistically significant difference between normal and dwarf scutellar measurements and none in kernel measurements. Therefore, the present study indicates very little or no recognizable influence of the gene  $d_1$  on those genes controlling scutellum and kernel growth in these early stages of ontogeny.

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#### ACKNOWLEDGMENTS

Acknowledgments are gratefully extended to the following persons: Dr. E. C. Abbe for helpful advice throughout the study and in preparation of the manuscript, and for provision of the maize kernels used in this study; Dr. J. F. Pelton for valuable assistance throughout the study; Mr. D. Baer for statistical advice; Mr. J. M. Olmsted for testing the maize kernels used in this work for variability and segregation, and for descriptions of the mature plants of the Station inbreds; Dr. B. Phinney for description of the Station inbred mature plants; and to Dr. S. C. Reed for criticism of the thesis manuscript.

TABLE I

Mathematical means for measurement of 50 excised scutella of each Station inbred.

Station Inbred	Length	Width	Depth
A21	6.90mm	4.48mm	3.29mm
A25	7.81mm	4.22mm	3.05mm
A172	7.64mm	5.15mm	3.85mm
A188	7.82mm	5.54mm	2.86mm

TABLE II

Comparison of scutellar size between normal and dwarf individuals.

Inbred Culture	Phenotype <sup>1</sup>	Number <sup>2</sup>	Scutellar Length			Scutellar Width		
			Mean	Standard Deviation	Probability <sup>3</sup>	Mean	Standard Deviation	Probability <sup>3</sup>
A21	N	80	7.56	0.591	3.2%	5.93	0.616	0.02%
	D	24	7.75	0.280		5.47	0.500	
A25	N	94	7.24	0.761	81.8%	5.30	0.648	67.4 %
	D	32	7.20	0.670		5.25	0.538	
A172	N	83	7.06	0.761	58.9%	5.20	0.331	68.9 %
	D	39	7.12	0.447		5.16	0.565	
A188	N	72	8.31	0.501	2.5%	6.46	0.655	19.7 %
	D	33	8.59	0.616		6.30	0.538	

<sup>1</sup> N = Normal, D = Dwarf.<sup>2</sup> Total number of individuals included in study.<sup>3</sup> Probability of the significant difference between means.

TABLE III

Comparison of kernel size between normal and dwarf individuals.

Inbred Culture	Phenotype <sup>1</sup>	Number <sup>2</sup>	Kernel Length			Kernel Width		
			Mean	Standard Deviation	Probability <sup>3</sup>	Mean	Standard Deviation	Probability <sup>3</sup>
A21	N	80	9.26	0.700	93.6%	7.01	0.538	39.0 %
	D	24	9.25	0.316		7.15	0.728	
A25	N	94	10.30	1.396	40.1%	7.53	1.126	42.4 %
	D	32	10.06	1.382		7.70	0.994	
A172	N	83	9.04	0.916	45.3%	8.48	0.883	2.2 %
	D	39	9.18	0.979		8.81	0.655	
A188	N	72	10.22	0.761	32.7%	9.33	0.932	77.2 %
	D	33	10.38	0.774		9.38	0.728	

<sup>1</sup> N = Normal, D = Dwarf.<sup>2</sup> Total number of individuals included in study.<sup>3</sup> Probability of the significant difference between means.