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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, Daubenmire, Potzger, and Billings served as Presidents of the Ecological Society of America.

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EXPRESSION OF THE GENE d_1 IN THE FIRST THREE LEAVES OF *ZEA MAYS* L.

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The present study is a continuation of investigations of expression of the gene dwarf-one (d_1) in the early stages of ontogeny in maize. An earlier study indicated no visible influence of the gene at the mature embryo stage (Pelton 1954), although expression appears as early after germination as the first stages of development of the mesocotyl (Hansen 1950, Hansen and Abbe 1943). The objective of the present study is to explore the expression of d_1 in those stages of seedling development which include maturation of the first three leaves.

History of the gene d_1 dates back to 1910 when the first mutant individuals were discovered in the Cornell breeding plots (Emerson 1912). These dwarfs were later described to be the result of a simple Mendelian recessive, located on the third chromosome (Emerson, Beadle, and Fraser 1935). An early quantitative study determined that the homozygous recessive dwarfs at maturity are shorter in stature and have shorter and wider leaves than do their normal sibs (Abbe, L. B. 1936). Since then there have been several studies on the morphogenetic effects of the gene (Abbe and Phinney 1940, 1942, Hansen and Abbe 1943, Phinney 1946, Hansen 1950, Olmsted 1951, Pelton 1954, Stein 1955). The present investigation concentrates on the expression of the gene in the seedling stage of ontogeny.

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MATERIALS AND METHODS

Source of Materials

The maize kernels used in the present study were produced by a morphogenetic project directed by Dr. E. C. Abbe of the University of Minnesota Department of Botany. Five generations of backcrossing of d_1 to University of Minnesota station inbred A188 assured a fairly homogeneous background for the mutant gene. The resulting segregating offspring were selfed in 1951. Two lots from sister plants of this 1951 planting were the basis of the present

experiments. A more detailed explanation of the breeding program is given in a previous paper (Pelton 1954).

Experimental Methods

Experiments were conducted in the Oberlin College greenhouse. Three plantings were made in April and May of 1953, yielding 91 normal and 31 dwarf plants upon which the present study was based. Before planting in standard flats filled with loam soil the kernels were soaked in distilled water for twenty-four hours at room temperature. Identification by phenotype of the homozygus recessive plants is certain by the tenth day after germination. Soon after the leaves appeared daily measurements of maximum length and width of the first three leaves of normal (D_1D_1, D_1d_1) and dwarf (d_1d_1) individuals were recorded. Measurements used in analysis were those at maturity of length and width of the three leaves. The dates of maturity of these dimensions were also included in the analysis. Maturity of the leaves was assumed to have been reached when dimensions remain unchanged for three successive days.

RESULTS

Leaf Length. Mean lengths of the first three seedling leaves (referted to as leaves 1, 2, and 3 tespectively) of dwarf and normal plants are presented in Table I and illustrated in Figure 1.

A marked shortness of the dwarf leaf when compared with the comparable leaf of its normal sib is apparent. The pattern of this difference is one of increasing relative expression of d_1 in successive leaves (Figure 1). The mean length in leaf 1 in the normals is 11.6 mm greater than that of the dwarfs, while leaves 2 and 3 of the normals average respectively 36.0 mm and 87.0 mm longer than the corresponding leaves to the dwarfs. Stated in terms of percentages, leaves 1, 2, and 3 of the dwarfs are 68 percent, 62 percent, and 51 percent, respectively, as long as the comparable leaves of the normals.

Although some of this increase in difference is due to the greater size of successive leaves, allowance was made for this factor. Proportional length increases between leaf 1 and 2 and that between leaves 2 and 3 of the dwarfs were calculated. The theoretical length increase of leaves 2 and 3 of the dwarfs could then be compared with actual measurements. Any decrease of length in the dwarfs below the proportionately expected increase of successive leaves relative to leaf 1, was considered an *extended expression* of d_1. Each of the 31 dwarfs was compared to every normal and the results of these many calculations averaged (Figure 2). The mean *extended expression* was 13.7 mm when comparing measurements of leaves 1 and 2 while the mean *extended expression* between leaves 2 and 3 increased to 21.6 mm. Since the *extended*

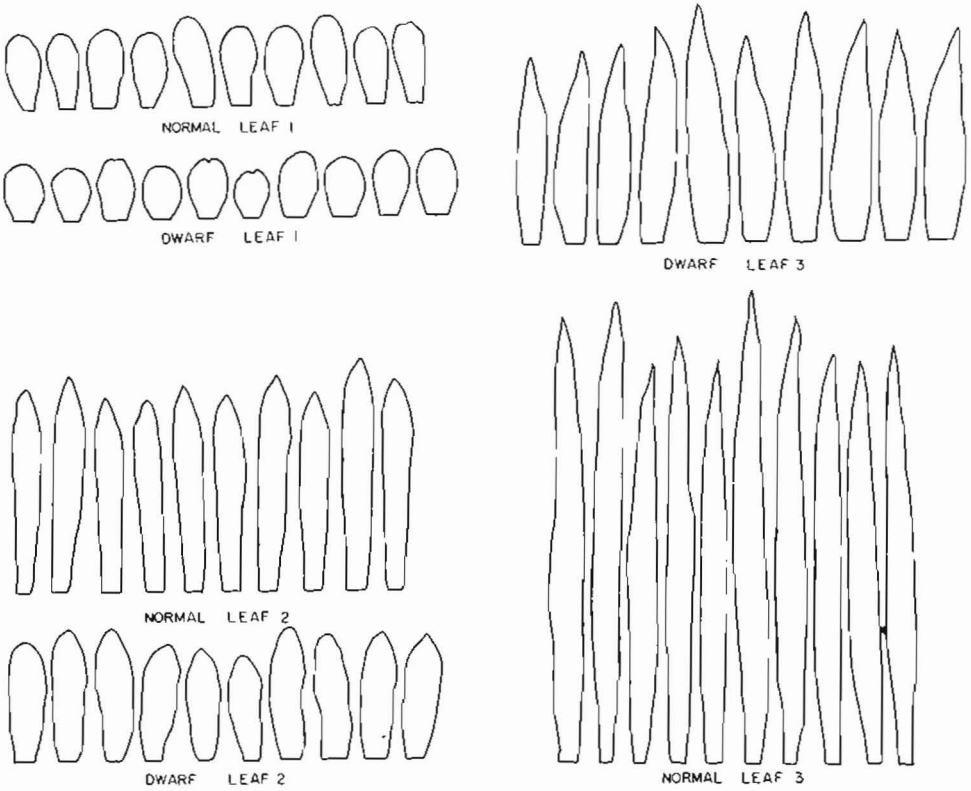

FIGURE 1

Tracings of mature leaves 1 through 3 from 10 dwarf and 10 normal plants.

expression of d_1 in leaves 2 and 3 relative to leaf 1 increases consistently, the trend is one of progressively increasing expression of d_1 in the first three leaves of maize.

Leaf Width. No consistent pattern of measurements such as was found in length seems to be present in the case of leaf width (Table I and Figure 1). In comparing measurements of dwarf and normal phenotypes the expected effect of d_1 is found in the slightly wider leaves of the homozygous recessives. The extent of difference varies, however, as leaf 1 of the dwarfs measures 2.0 mm wider than its normal sibs on the average, while the mean width of leaf 2 in the dwarf is 1.1 mm greater than that of the normals, and leaf 3 dwarfs are 3.8 mm wider than the normals on the average. Thus, the average difference between dwarfs and normals in leaf 2 is slightly less than that of leaf 1, while leaf 3 dwarf and normal differences are greater than those of

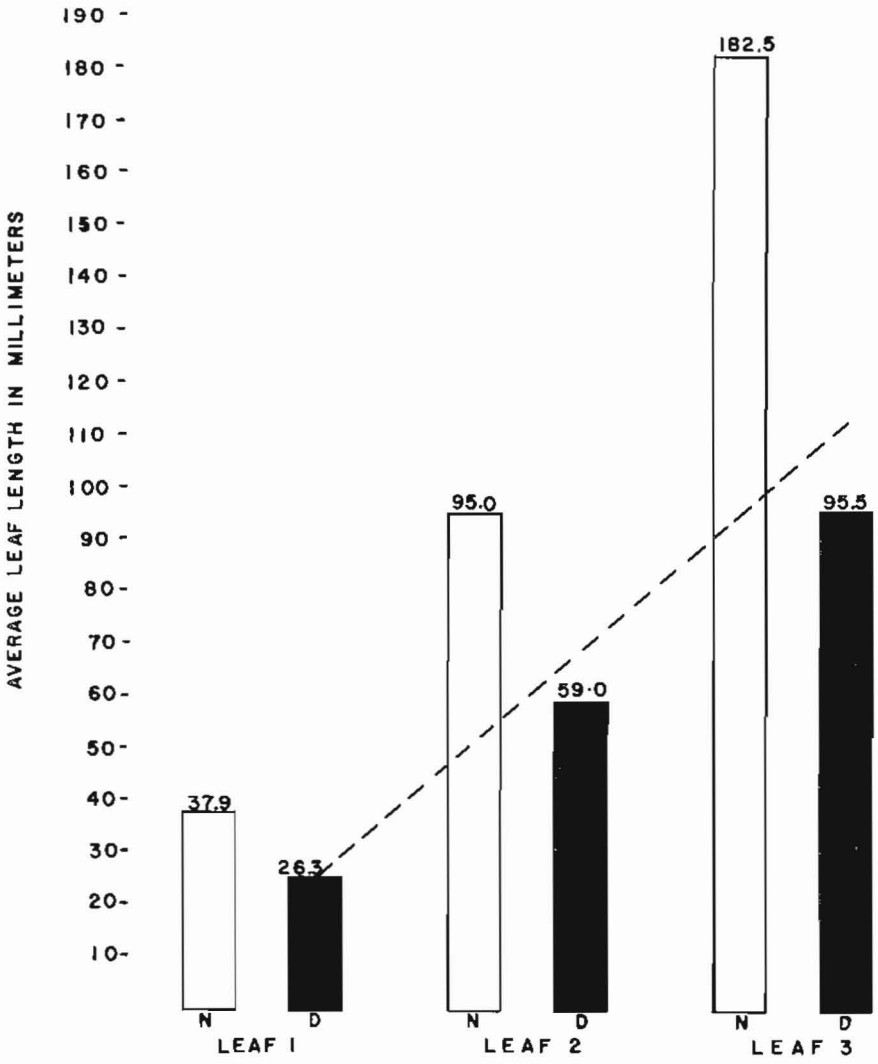

FIGURE 2

Mean length of dwarf and normal leaves 1 through 3. *Extended expression* is shown by the difference between the actual mean of the dwarf as recorded at the top of the bar and the calculated expected proportional increase of successive leaves as represented by the dotted line.

TABLE I

Average length and width of leaves 1 through 3 of dwarf and normal sibs, based on 91 normal and 31 dwarf plants.

	Leaf Length in Millimeters		Leaf Width in Millimeters	
LEAF	Mean and Standard Error	Difference between means of normal and dwarf plants	Mean and Standard Error	Differenee between means of normal and dwarf plants
LEAF 1				
normal	37.9± .56	11.6	15.7±.19	2.0
dwarf	26.3± .82		17.7±.21	
LEAF 2				
normal	95.0±1.6	36.0	14.8±.13	1.1
dwarf	59.0±1.8		15.9±.26	
LEAF 3				
normal	182.5±1.6	87.0	14.6±.24	3.8
dwarf	95.5±1.9		18.4±.34	

TABLE II

Maturation dates of length and width of the leaves 1 through 3 of dwarf and normal phenotypes expressed as average days after planting.

	Leaf length maturity date, in average number of days after planting			Leaf width maturity date, in average number of days after planting		
LEAF	Normal	Dwarf	Mean Differenee	Normal	Dwarf	Mean Difference
Leaf 1	8.7	9.1	0.4	8.4	8.7	0.3
Leaf 2	11.4	12.1	0.7	10.8	10.7	0.1
Leaf 3	14.8	16.6	1.8	14.3	14.5	0.2

leaves 1 or 2. Leaf 1 of the normals averages 88 percent as wide as that of the dwarfs, leaf 2 is 93 percent of the dwarfs, while the width of leaf 3 of the normals is 70 percent that of the dwarfs.

Maturation Dates. Table II gives the avetage maturation dates in length and width of both normal and dwarf plants. Average maturation dates in length show a pattern of increasing difference. Leaf 1 in the dwarfs matures in length 0.4 days later than their normal sibs on the average. Maturity of the dwarfs in length of leaf 2 averages 0.7 days later than its normal sibs. Difference between maturity of dwarf and normal sibs increases to a average of 1.8 days for leaf 3, in which the dwarf is again the slower to mature in length.

On the other hand, maturation dates in leaf width show no consistent trend. Leaf 1 of the dwarfs matures on the average 0.3 day after the leaves of their normal sibs have attained maximum width. In leaf 2 the dwarfs average 0.1 day earlier in width maturity, while in leaf 3 the dwarfs average 0.2 days later than their normal sibs.

DISCUSSION

Although in the present study expression of d_1 is evident in the seedling leaves, no expression of the gene has been observed very early in ontogeny. In the mature embryo normal and dwarf scutella do not differ significantly in their measurements (Pelton 1954). Soon after germination, however, expression of d_1 has been obsetved in a seedling organ. Hansen and Abbe report that homozygous recessive plants have shorter and wider mesocotyls than do their normal sibs (Hansen 1950, Hansen and Abbe 1943). Shortness of the dwarf mesocotyl is mainly the result of fewer cells having been formed than in its normal sibs. The increased mesocotyl width of the dwarfs as compared to the normals, however, is due to the slightly wider cells of the dwarf mesocotyl.

The present study adds quantitative data on expression of d_1 in the seedling leaves to the above investigations of d_1 in the eatlier stages of ontogeny. Although the time of leaf initiation of the dwarf and normal phenotypes was found to be similar in the first three leaves by Stein (1955), these same leaves show phenotypic differences at maturity. Expression of the gene, therefore, has come after leaf initiation. In leaves 1 thtough 3, as in the later formed leaves, length of the dwarf leaf is less than that of its normal sib while the width of the dwarf leaf is greater than that of the normal.

Analysis of the data on leaf length indicates that expression of d_1 is increasingly greater in the successive seedling leaves. Consecutive leaves of the dwarfs are progressively shorter as compared to their normal sibs. The tetm *extended expression* is suggested to describe the increased expression of the gene in successive leaves relative to its expression in leaf 1. Although the number of

successive leaves of maize included in this study is too small to come to definite conclusions, the *extended expression* of d_1 seems to consistently increase in the series of leaves measured. That is, there is a progressive expression of d_1 in the three successive leaves. The possibility that this trend is continued in leaves beyond the third is suggested by the study of Phinney (1946) on the eighth leaf of field-grown maize. In this case the eighth dwarf leaf was only 40 percent as long as the eighth leaf of the normal, in contrast with the present study in which the third dwarf leaf was 51 percent as long as the third leaf of the normal. Comparisons with the present study are difficult however, because of the lack of environmental and genetic uniformity between the two investigations.

It is interesting to observe a consistently progressive expression also in the time required for cessation of length growth in the first three leaves. The lack of any consistent trend in leaf width, either from actual measurement or for maturation dates, is puzzling, however, in comparison with the definite trends for leaf length. It therefore seems that in the seedling leaves of maize homozygous for d_1 length of each successive leaf takes progressively more time to attain relatively less growth than in the case of the normal sibs. On the other hand, width of the successive leaves of dwarf plants becomes greater in essentially the same time period required for the normals, and without showing any consistent trend of increasing width.

The relative importance of differential cell division versus cell enlargement in explaining these results is as yet unknown. Hansen and Abbe's work on the mesocotyl (Hansen 1950, Hansen and Abbe 1943) has indicated that reduced length of the dwarf mesocotyl results from a slower rate of cell division, while increased mesocotyl width of the dwarf is a consequence of greater cell enlargement. Whether or not the same pattern occurs in the seedling leaves as occurs in the mesocotyl will require further study. In addition, the physiological basis for the observed changes and the mode of action of d_1 could be probed as in the 1953 Harris work and the current work in the University of California at Los Angeles laboratories of Dr. B. Phinney.

SUMMARY

The expression of a mutant gene, d_1, is analyzed quantitatively for the first three leaves in *Zea mays L.* Maximum length and width as well as dates of maturity of these dimensions in the seedling leaves were recorded for 31 dwarf and 91 normal sibs. Analysis of the data on leaf length shows progressively increasing shortness of successive dwarf leaves as compared to their normal sibs. The expression of the gene d_1 in leaf length is therefore increasingly greater in successive seedling leaves. This progressive trend of gene expression is also present in dates of maturity of leaf length since dwarf leaves mature later in

length than do those of their normal sibs. On the other hand, although the three seedling dwarf leaves all average wider than those of the normals, leaf width does not show a progressive or consistent trend as does leaf length. Nor do maturity dates of leaf width show the consistent trend that is shown by maturation of leaf length.

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