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Genetic Studies of Induced Mutants in *Melilotus alba*. I. Short-Internode Dwarf, Curled Leaf, Multifoliolate Leaf, and Cotyledonary Branching

B. G. Gengenbach

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

H. J. Gorz

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I. Short-Internode Dwarf, Curled Leaf, Multifoliolate Leaf, and Cotyledonary Branching[1]

B. G. Gengenbach, F. A. Haskins, and H. J. Gorz[2]

ABSTRACT

Information obtained from the F_1, F_2, and F_3 generations of crosses between the normal annual *M. alba* progenitor line and the four mutants, short-internode dwarf, curled leaf, multifoliolate leaf, and cotyledonary branching, indicates that each character is conditioned by a single pair of alleles. The multifoliolate leaf character is dominant over the normal phenotype; the other three mutant characters are recessive. The symbols *dw*, *cl*, *Mf*, and *cb* are proposed as designations for the respective mutant genes.

Additional index word: Sweetclover.

INHERITANCE studies in white-flowered sweetclover, *Melilotus alba* Desr., have dealt with a relatively small number of mutations, most of which occurred spontaneously. The 1965 review of Smith and Gorz (10) lists only 19 mutant traits for which the mode of inheritance has been elucidated. Since 1965 an additional mutant gene, responsible for the unifoliolate leaf-cauliflower inflorescence phenotype, has been reported by Goplen (3). The need for added genetic markers in this species is obvious.

Within the past 15 years several workers have succeeded in isolating new mutant strains of *M. alba* following the use of ionizing radiation or chemical mutagens. Mutagenic work on this species was started in the early 1950's when Micke (7) began a series of experiments in which dry seeds were irradiated with X rays or thermal neutrons. From this and other studies of Micke and of Scheibe and Hülsmann (8), who utilized chemical mutagens, some 690 independently occurring mutations had been identified by 1966 (9). Very few of these mutants have been subjected to genetic analysis.

A recent report from this laboratory (6) deals with the isolation of numerous mutants in an annual strain of *M. alba* in the second generation following treatment of seeds with ethyl methanesulfonate. The present paper deals with the inheritance of four of these mutants.

MATERIALS AND METHODS

All plants used in this study were derived from an annual strain of *M. alba* which originated as a plant introduction (P.I. 165,554) from India. Plants of this strain are relatively short in stature, autogamous, and have a short life cycle; hence they are well suited for culture in growth chambers. The plants were grown in chambers at 25 C and approximately 70% relative humidity, with continuous cool white fluorescent light at about 1,000 ft-c. Under these conditions normal plants attain a height of 35 to 50 cm and produce ripe seeds within about 8 weeks after planting. Branches, if present, are small in number and short in length. Leaves are trifoliolate with obovate, moderately serrated leaflets.

The induction and subsequent isolation of mutants have been described (6). The four mutant characters chosen for study are short-internode dwarf, curled leaf, multifoliolate leaf, and cotyledonary branching (Fig. 1). Plants for crossing with the normal annual *M. alba* parent were selected from M_4 lines of these mutants. These lines resulted from two generations of selfing following isolation of the mutants (three generations of selfing following treatment with the mutagen). Under growth chamber conditions the four mutant lines exhibit the following distinct phenotypes:

Short-internode dwarf plants have about the same number of nodes as normal plants, but they attain a height of only 5 to 10 cm. Petiole length and leaflet size are reduced slightly. Plants do not branch; they typically appear very compact and sturdy.

Curled leaf plants are characterized by curled leaflets and twisted or curved stems, petioles, and flower parts. Leaflet edges curl up and toward the midrib, producing a canoe-shaped appearance. Random curvature of the stem, first in one direction for a short distance and then in another, tends to result in a semiprostrate growth habit. Petiole curvature is typically less pronounced than stem curvature. Filaments and styles are usually slightly twisted, and petal curvature is greater than normal.

Multifoliolate leaf plants have leaves composed of one to six leaflets of varying morphology, ranging from moderately to severely serrated, and from obovate or rectangular to mere bracts. The size, shape, and number of leaflets comprising one leaf vary considerably on a single plant. A few apparently normal trifoliolate leaves occur on some plants.

The cotyledonary branching mutant (also referred to as the branched mutant) is characterized by the formation of a single branch in the axil of each cotyledon. These branches from the cotyledonary node become visible 2 to 3 weeks after seedling emergence. Branches then develop sequentially up the stem from other leaf axils. The growth rate of this mutant is faster than normal for the first 2 weeks, but it decreases at about

[2] Formerly Graduate Assistant in Agronomy, University of Nebraska, (now graduate student in the Agronomy Department, University of Illinois); Bert Rodgers Professor of Agronomy, University of Nebraska, Lincoln 68503; and Research Geneticist, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Lincoln, Nebr., respectively.
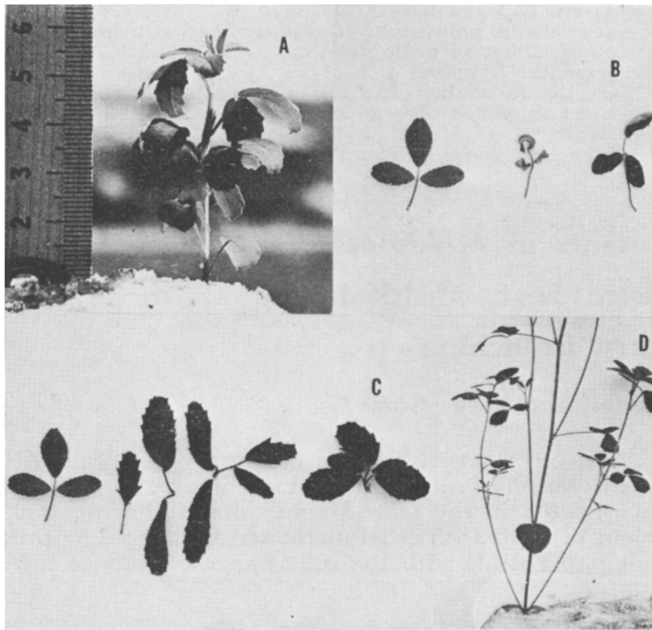
Fig. 1. (A) Short-internode dwarf plant 30 days after planting. (B) Leaf from a normal plant (left) and two leaves from a curled leaf plant. (C) Leaf from a normal plant (left) and four leaves from one multifoliolate leaf plant. (D) Basal portion of a cotyledonary branching plant 38 days after planting.

the time branching starts. Mature plants are shorter (1/2 to 2/3 as tall) and bushier than normal.

Plants were grown from hand-scarified seeds, planted in 1-pint plastic coated milk cartons filled with a mixture of vermiculite, soil, and sand (6:4:1 by volume), and covered with a 1-cm layer of silica sand mixed with Orthocide[3] (0.11 g Orthocide per liter of sand). The cartons were placed in plastic trays and watered by subirrigation. Parental, F_1, and F_2 lines were planted at the rate of five seeds per carton; F_3 lines were planted at the rate of nine seeds per carton.

Crosses between normal and mutant plants were made by first emasculating flower buds of the female parent, and then immediately pollinating these buds with pollen from the appropriate male parent. Reciprocal pollinations were made initially, but if and when several seeds had been obtained from a given cross, attempts to effect the reciprocal cross were abandoned.

Seeds obtained from each cross were planted, and the resulting F_1 plants were compared to plants of the parental lines with respect to morphology, general vigor, and seed set. F_1 plants were allowed to self-pollinate, and from each plant 115 seeds were planted to produce the F_2 generation. An exception to this procedure occurred in the case of two F_1 plants from the cross, normal $\times$ multifoliolate leaf, from which only 30 and 60 seeds were available for planting. Plants in the segregating F_2 populations were classified phenotypically. On the basis of the F_1 and F_2 data, a tentative hypothesis was formulated concerning the mode of inheritance of the mutant character. Chi-square (χ^2) tests were applied to the F_2 data for each family and, where applicable, for pooled families to determine whether the data fit the hypothesis. Heterogeneity χ^2 values also were calculated.

Each tentative hypothesis was tested further in the F_3 generation. The progenies of several F_2 plants classified as recessive were planted to determine whether the putative recessive condition bred true, and the progenies of at least 20 F_2 plants of the dominant phenotype also were checked for segregation. In this progeny testing, nine seeds of each recessive F_2 plant, and in most cases, 18 seeds of each dominant F_2 plant were planted

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[3] Mention of a specific product is for identification only and does not imply endorsement by the U. S. Department of Agriculture.

for observation. Chi-square tests were applied to the following: (a) distribution of normal and mutant plants in each segregating F_3 family, (b) the pooled data for all segregating F_3 families, (c) heterogeneity among the segregating F_3 families, and (d) distribution of segregating and nonsegregating families from dominant F_2 plants. Numbers were small (less than five) in some cases, particularly in the recessive class of some F_3 families, but no correction was applied in the calculation of χ^2 values.

RESULTS AND DISCUSSION

Short-Internode Dwarf

Hybrid seeds were obtained from the cross, short-internode dwarf $\times$ normal, but none from the reciprocal cross. The three F_1 plants grown were phenotypically normal, indicating that the dwarf trait is recessive. The ratio of normal to short-internode dwarf plants in the F_2 generation was approximately 3:1 (Table 1). A single gene pair was proposed to explain the observed inheritance pattern, with normal completely dominant over the dwarf growth habit.

The F_3 results supported this hypothesis. The frequencies of normal and dwarf plants in segregating F_3 families fit a 3:1 ratio (Table 2); and a 2:1 ratio of segregating to nonsegregating F_3 families from normal F_2 plants was observed (Table 3). The progenies of five dwarf F_2 plants were all dwarf, as expected for a recessive character.

On the basis of these results, it was concluded that the short-internode dwarf character is indeed controlled by one gene pair with normal height completely dominant over dwarf. The symbols Dw and dw are suggested for the dominant and recessive alleles, respec-

Table 1. Chi-square tests for goodness-of-fit to a 3:1 ratio for the distributions of normal and mutant plants in the F_2 generation of crosses between normal and mutant plants.

	Mutant character			
Statistic	Short-internode dwarf	Curled leaf	Multi-foliolate leaf	Cotyledonary branching
No. of families	3	3	2	1
Class distrib. (pooled) Normal:Mutant	203:61	199:74	17:61	75:28*
Sum of family χ^2 values	2.90	1.94	1.28	---
χ^2, pooled data	0.51	0.65	0.43	0.26
P value	0.25<P<0.50	0.25<P<0.50	0.50<P<0.75	0.50<P<0.75
Heterogeneity χ^2	2.39	1.29	0.85	---
P value	0.25<P<0.50	0.50<P<0.75	0.25<P<0.50	---

* The normal class includes individuals originally classified as intermediate (see text).

Table 2. Chi-square tests for goodness-of-fit to a 3:1 ratio for the distributions of normal and mutant plants in the F_3 generation of crosses between normal and mutant plants.

	Mutant character			
Statistic	Short-internode dwarf	Curled leaf	Multi-foliolate leaf	Cotyledonary branching
No. of families	15	14	17	23
Class distrib. (pooled) Normal:Mutant	192:50	134:33	54:148	284:103
Sum of family χ^2 values	12.66	8.02	13.20	37.91
χ^2, pooled data	2.43	2.44	0.32	0.54
P value	0.10<P<0.25	0.10<P<0.25	0.50<P<0.75	0.25<P<0.50
Heterogeneity χ^2	10.23	5.58	12.88	37.37
P value	0.50<P<0.75	0.95<P<0.975	0.50<P<0.75	0.01<P<0.025

Table 3. Chi-square tests for goodness-of-fit to a 2:1 ratio for the distributions of segregating and nonsegregating F_3 families from dominant F_2 plants.

	F_3 families observed			
Mutant character	Segregating	Nonsegregating	Chi-square	P value
Short-internode dwarf	15	5	0.63	0.25<P<0.50
Curled leaf	14	6	0.10	0.75<P<0.90
Multifoliolate leaf	17	3	3.03	0.05<P<0.10
Cotyledonary branching	23	15	0.64	0.25<P<0.50

tively. The short-internode dwarf character differs phenotypically from other dwarf characters which have been reported in *M. alba* (1, 2, 4, 5), but it is similar to these other dwarfs in its monogenic recessive inheritance.

Curled Leaf

Crosses between the curled leaf mutant and normal plants were successful when the mutant was used as the male parent. Three F_1 plants were obtained from this cross, and all were normal in morphology and seed set. Plants in the F_2 generation were readily classified as either normal or curled leaf; no other phenotypes were found. The observed F_2 and F_3 frequencies of normal and mutant plants agreed well with a 3:1 ratio (Tables 1 and 2). Among the progenies of phenotypically normal F_2 plants, a ratio of approximately two segregating families to one non-segregating family was observed (Table 3). Plants grown from selfed seed of four curled leaf F_2 plants were all mutant in phenotype. Evidently the curled leaf character, like the short-internode dwarf, is recessive and monogenic. The designation proposed for the curled leaf allelic pair is Cl/cl.

Multifoliolate Leaf

Attempts to use multifoliolate leaf plants as the female parent in crosses with normal plants were unsuccessful. Five F_1 seeds obtained from the cross, normal $\times$ mutant, produced multifoliolate plants, suggesting that the mutant character is dominant. Progenies of three of the five F_1 plants segregated for a leaf mottling character as well as for multifoliolate leaves. Progenies of the other two F_1 plants, which appeared to segregate only for the multifoliolate character, were used in further studies. Seed set on these two plants was less than half the normal level; thus the F_2 numbers are relatively small (Table 1). Nevertheless, a good fit to the expected ratio of three mutant to one normal was obtained. Similarly a ratio of approximately 3:1 was observed for the mutant and normal individuals in segregating F_3 families (Table 2). The ratio of segregating to nonsegregating F_3 families from multifoliolate F_2 plants was higher than expected, but the χ^2 value was not significant at the 0.05 probability level (Table 3). F_3 progenies from normal F_2 plants were uniformly normal in phenotype.

All available evidence supports the conclusion that the multifoliolate character is controlled by one gene pair, and that the mutant phenotype is dominant over the normal trifoliolate condition. The designation Mf/mf is proposed for this allelic pair. Reports of other *M. alba* mutants resembling this one have not been found. However, Bingham (E. T. Bingham, 1965. Inheritance of multifoliolate leaves in diploid alfalfa. Agron. Abstr. p. 8) reported that a recessive gene is responsible for a multifoliolate mutant of diploid *Medicago sativa* L., with two additive genes influencing the variability of expression.

Cotyledonary Branching

A single F_1 plant, intermediate in phenotype between the two parents, was obtained from a cross of branched $\times$ normal. No branches arose from the cotyledonary axils, but branch formation did occur in several of the higher leaf axils. In the F_2 generation, plants were classified into three categories: normal, intermediate, and branched. The intermediate class included plants whose branching resembled that of the F_1, as well as some plants which had poorly developed branches arising from the cotyledonary node. Accurate assignment of very young F_2 plants to the three classes was not possible, but classification was readily accomplished after the plants had reached an age of about 4 weeks. The F_2 distribution, 26 normal:49 intermediate:28 branched, suggested a 1:2:1 ratio. This distribution, together with the intermediate phenotype of the F_1, prompted speculation that intermediate plants might be heterozygous. However, F_3 results failed to verify this speculation. Of six F_3 families from normal F_2 plants, four bred true for normal and two segregated. Among 32 F_3 families from intermediate F_2 plants, 21 segregated and 11 were completely normal. Considering the 38 F_3 families from normal and intermediate F_2 plants together, 23 segregated and 15 did not, providing a reasonably good fit to a 2:1 ratio (Table 3).

Clearly the normal and intermediate F_2 classes could not be reliably classified as homozygous and heterozygous genotypes, respectively. Consequently the normal and intermediate classes were combined in the "normal" category in the F_2 (Table 1), and no attempt was made to distinguish between normal and intermediate in the F_3. Only branched plants were observed in six F_3 families from branched F_2 plants.

The F_2 ratio of normal to branched is close to 3:1 (Table 1) as is the ratio based on the 23 segregating F_3 families (Table 2). However, the heterogeneity χ^2 value exceeds that representing the 0.05 probability level. One of the F_3 families included 16 normal and 15 branched individuals. This family contributed 9.04 to the sum (37.91) of χ^2 values for all F_3 families. Except for this family, the χ^2 value based on pooled data would have been 0.015 ($0.90 < P < 0.95$), and the heterogeneity χ^2 would have been 28.86 ($0.10 < P < 0.25$).

On the basis of the available data, it is reasonable to postulate that the branched character is recessive and is governed by one major gene pair. The designation Cb/cb is proposed for this pair of alleles. The lack of uniformity among the F_2 plants carrying the Cb allele might have resulted from interaction of this allele with other genes introduced from the branched parent. However, no definite evidence was obtained on this point.

Future Use of Mutants

All four of the mutant strains described in this report have good plant vigor and adequate seed production for line maintenance. Also, each of the mutant phenotypes is readily distinguished from normal. Because of these attributes, the four mutants hold much promise for further use in genetic studies in *M. alba*. The mutant strains also might be used in biochemical investigations. In particular, the short-internode dwarf and cotyledonary branching mutants might be useful in studies of growth-regulating compounds in *Melilotus*.

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