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Genetic Studies of Induced Mutants in *Melilotus alba.* IV. Inheritance and Complementation of Six Additional Chlorophyll-deficient Mutants

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Genetic Studies of Induced Mutants in *Melilotus alba*. IV. Inheritance and Complementation of Six Additional Chlorophyll-deficient Mutants[1]

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ABSTRACT

Six ethyl methanesulfonate-induced chlorophyll-deficient mutants of *Melilotus alba* Desr. were studied. Each of the mutants behaved as a monogenic recessive. Complementation analysis revealed that the genes from five of the mutants were nonallelic to each other and to the previously reported mutants ch_4, ch_5, ch_6, and ch_7. Proposed symbols for the five genes are ch_8, ch_9, ch_{10}, ch_{11}, and ch_{12}. No linkage was detected between ch_5 and ch_7, ch_7 and M_f (multifoliolate leaf), ch_8 and ch_4, ch_8 and ch_5, ch_8 and ch_6, and ch_8 and ch_7.

Additional index words: **Sweetclover, Ethyl methanesulfonate.**

IN a recent report from this laboratory (1), studies of the inheritance and complementation of six viable, chlorophyll-deficient mutants in an annual form of sweetclover (*Melilotus alba* Desr.) were described. These and numerous other mutants were isolated in the second generation after treatment of seeds with ethyl methanesulfonate (4). Five of the chlorophyll-deficient mutants behaved as monogenic recessives. In the sixth mutant, two independent recessive alleles were responsible for the observed phenotype. Complementation analysis revealed that five of the seven genes detected in the mutants were nonallelic. Four of the genes, designated ch_4, ch_5, ch_6, and ch_7, conditioned chlorophyll-deficient phenotypes. The fifth gene, ch_v, was associated with the presence of dark-green venation in chlorophyll-deficient leaves.

The present paper deals with an extension of the above studies to include six additional chlorophyll-deficient mutants. The mode of inheritance of each mutant was determined; complementation was studied within this group of six, as well as with the mutants studied by Gengenbach, Gorz, and Haskins (1).

MATERIALS AND METHODS

The six chlorophyll-deficient mutants used in this study were derived from the same source as previously described (5). Growth conditions and procedures also were essentially the same as described for the morphological mutants (5). In addition to crosses of normal × mutant for studies of the inheritance of each mutant, tests for complementation were made by intercrossing the six mutants in most of the possible combinations, and by crossing each of the six with ch_4, ch_5, Ch_6, and ch_7 mutant lines. The system used for color classification has been described by Gengenbach et al. (1). The color classes occurring in this study were: #3, yellow-green; #4, light green; and #5, dark (normal) green. Color differences were most apparent when plants were 2 to 3 weeks old; hence, colors were classified when seedlings had reached this age. Brief descriptions of the mutant lines follow (color class numbers are based on classification of fully expanded leaves):

U369 (Color Class #4). Leaves were yellow-green when very young and light green when fully expanded, with midribs lighter in color and distinct from the rest of the leaflet. Unifoliolate leaves were very light green, whereas stems were pink (much lighter than the normal red color). Plants were shorter and less vigorous than normal with reduced seed set.

U370 (Color Class #3). Newly emerged leaves were light yellow but become yellow-green as leaves reached full expansion. Despite the yellowish color, these plants were almost normal in height and vigor.

U371 (Color Class #4). Unifoliolate leaves were light green. Trifoliolate leaves were yellow-green when newly emerged, turning to light green at full expansion and gradually darkening to nearly normal green at maturity. Flowering and seed production were near normal.

U372 (Color Class #4). Leaves were light green from emergence to maturity. Appearance, vigor, and seed set were near normal.

U373 (Color Class #4). Unifoliolate leaves were yellow-green. Trifoliolate leaves were initially yellow-green but changed to light green. Plants lacked vigor; flowering and seed set were reduced.

U374 (Color Class #4). Unifoliolate leaves were yellow-green Light-green color of trifoliolate leaves remained constant.

Homozygous lines with the following genes for chlorophyll-deficiency studied by Gengenbach et al. (1), were classified for color in the present study as follows: Q839 (ch_4, #3); Q843 (ch_5, #4); Q844 (ch_6 #4); and Q856 (ch_7, #4). These lines were crossed to the six mutant lines listed above, to study complementation of the genes involved.

RESULTS AND DISCUSSION

F_1 plants were obtained from reciprocal crosses of U371, U373, and U374 with the normal annual, but crosses involving the remaining three mutants succeeded only when the mutant was used as the female parent. Each of the 28 F_1 plants was dark green, indicating that all six mutants were conditioned by recessive genes. Ratios of normal to chlorophyll-deficient plants in the F_2 and segregating F_3 progenies approximated 3:1 for each mutant, although significant χ^2 values were obtained for one F_2 and two F_3 progenies (Table 1).

Each of the ratios differing significantly from 3:1 was found to be markedly deficient in the mutant class. Differential shattering of seeds produced on heterozygous plants (with seeds of the homozygous recessive genotype shattering first) could have caused the aberrant ratios. Shattering of this type was found in a previous study (3). Phenotypically mutant F_2 plants from each of the six lines bred true in the F_3 generation. The classification of F_3 families from normal F_2 plants provided a satisfactory fit to the 2:1 ratio expected in monogenic inheritance in all but two of the families observed (Table 2). Despite the observed deviations, the most reasonable interpretation of the

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Table 1. Chi-square tests for goodness-of-fit to a 3:1 ratio in F_2 and F_3 segregating populations from crosses of six chlorophyll-deficient mutants to normal plants.

Mutant line	Generation*	Classification Normal:mutant	χ^2	P range
U369	F_2(4)	298:100	0.003	0.95
	F_3(13)	250: 52	9.753	<0.005
U370	F_2(8)	606:199	0.034	0.75 -0.90
	F_3(14)	240: 65	2.213	0.10 -0.25
U371	F_2(4)	452:114	7.126	0.005-0.01
	F_3(4)	68: 23	0.004	0.90 -0.95
	(13)	187: 75	1.837	0.10 -0.25
	(9)	156: 44	0.960	0.25 -0.50
U372	F_2(4)	442:146	0.009	0.90 -0.95
	F_3(16)	255: 90	0.217	0.50 -0.75
U373	F_2(3)	495:180	1.000	0.25 -0.50
	F_3(13)	202: 66	0.020	0.80 -0.90
U374	F_2(5)	694:217	0.677	0.25 -0.50
	F_3(9)	124: 27	4.082	0.025-0.05
	(12)	233: 49	8.742	<0.005
	(6)	115: 32	0.819	0.25 -0.50
	(17)	321: 78	0.010	0.90 -0.95

* The numbers in parentheses refer to the number of families included in the pooled data. In the F_3, families originating from a single F_1 plant were pooled.

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

Mutant line	F3 families observed* Segregating	Nonsegregating	χ^2	P range
U369	13	7	0.25	0.75-0.90
U370	14	7	0.000	>0.99
U371	4	15	17.808	<0.005
	13	11	1.688	0.10-0.25
	9	6	0.300	0.50-0.75
U372	16	5	0.857	0.25-0.50
U373	13	7	0.025	0.75-0.90
U374	9	8	1.437	0.10-0.25
	12	2	2.291	0.10-0.25
	6	5	0.723	0.25-0.50
	17	0	8.508	<0.005

* F_3 families from a single F_1 plant are grouped.

available evidence is that each of the six chlorophyll-deficient mutants resulted from a monogenic alteration, with the chlorophyll-deficient phenotype being recessive to the dark green of normal plants.

F_1 plants from all crosses for the assessment of gene complementation, except the cross of ch_5 (Q843) × U374, were normal green (Table 3). From these results, it appears that the five mutant genes from U369, U370, U371, U372, and U373 (designated in Table 3 as ch_8, ch_9, ch_{10}, ch_{11}, and ch_{12}, respectively) were nonallelic to each other and to the chlorophyll-deficient mutants ch_4, ch_5, ch_6, and ch_7 reported by Gengenbach et al. (1). Noncomplementation in the cross ch_5 (Q843) × U374 suggests that mutant U374 was allelic to ch_5 and therefore, was nonallelic to U369, U370, U371, U372, and U373. It is noteworthy that the only allelic chlorophyll-deficient mutations detected in the earlier (1) or the present study were those apparently allelic to ch_5.

Analysis of F_2 results from two-factor crosses indicated good fits to a dihybrid ratio when the following combinations were considered: ch_5 and ch_7; ch_7 and M_f [gene for multifoliolate leaves (2)]; ch_8 and ch_4; ch_8 and ch_5; ch_8 and ch_6; and ch_8 and ch_7. Thus, the two genes in each combination appeared to be independently inherited.

Table 3. Intercrosses among 10 chlorophyll-deficient mutant of annual *M. alba* to test for gene complementation.

Mutants crossed*	No. F_1 plants	Mutant genotypes
ch_4 × U369	5	ch_4ch_4 × ch_8ch_8
× U370	18	× ch_9ch_9
× U371	5	× $ch_{10}ch_{10}$
× U372	12	× $ch_{11}ch_{11}$
× U373	13	× $ch_{12}ch_{12}$
× U374	7	× ch_5ch_5
ch_5 × U369	7	ch_5ch_5 × ch_8ch_8
× U370	9	× ch_9ch_9
× U371	5	× $ch_{10}ch_{10}$
× U372	5	× $ch_{11}ch_{11}$
× U373	3	× $ch_{12}ch_{12}$
× U374†	32	× ch_5ch_5
ch_6 × U369	9	ch_6ch_6 × ch_8ch_8
× U370	11	× ch_9ch_9
× U371	7	× $ch_{10}ch_{10}$
× U372	13	× $ch_{11}ch_{11}$
× U373	9	× $ch_{12}ch_{12}$
× U374	11	× ch_5ch_5
ch_7 × U369	5	ch_7ch_7 × ch_8ch_8
× U370	6	× ch_9ch_9
× U371	5	× $ch_{10}ch_{10}$
× U372	4	× $ch_{11}ch_{11}$
× U373	8	× $ch_{12}ch_{12}$
× U374	8	× ch_5ch_5
U369 × U370	4	ch_8ch_8 × ch_9ch_9
× U371	6	× $ch_{10}ch_{10}$
× U372	7	× $ch_{11}ch_{11}$
× U373	6	× $ch_{12}ch_{12}$
U370 × U371	5	ch_9ch_9 × $ch_{10}ch_{10}$
× U372	9	× $ch_{11}ch_{11}$
× U373	5	× $ch_{12}ch_{12}$
U371 × U372	3	$ch_{10}ch_{10}$ × $ch_{11}ch_{11}$
× U373	7	× $ch_{12}ch_{12}$
U372 × U373	4	$ch_{11}ch_{11}$ × $ch_{12}ch_{12}$

* The arrangement of parents in each cross does not imply that the mutant listed first was the female parent. Reciprocal crosses were made in some but not in all cases.
† The only cross yielding chlorophyll-deficient F_1 progeny.

A total of nine single-gene chlorophyll-deficient mutants have now been reported in the group of mutants induced in an annual strain of *M. alba* by Kleinhofs, Gorz, and Haskins (4). Each of these mutants is sufficiently vigorous to permit good growth in a greenhouse or growth chamber, seed production is adequate for line maintenance, and each mutant is readily distinguished from the normal. These attributes contribute to the usefulness of these mutant lines in various investigations of the photosynthetic apparatus in sweetclover. A recent study on the contents of chlorophylls a and b in these lines will be published in a subsequent paper.

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