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

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

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ABSTRACT

Six ethyl methanesulfonate-induced mutants of *Melilotus alba* Desr. were studied. Five of the mutants behaved as monogenic recessives. In the sixth mutant, two independent recessive alleles were responsible for the observed phenotype. Of these two genes, one had a phenotypic effect similar to the five single-gene mutants. The other (the veined gene) caused a chlorophyll deficiency in which the leaf veins were darker in color than the tissue between the veins. Complementation analysis revealed that five of the seven genes detected in the mutants were nonallelic. Suggested designations for the five genes are ch_4, ch_5, ch_6, ch_7, and ch_v.

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

GENE mutations influencing the green coloration of photosynthetically active parts are among the most common spontaneous or induced alterations arising in higher plants. These are usually referred to collectively as chlorophyll mutants. They range from lethal to semilethal or completely viable types having white, yellow, or pale green leaves and stems. Many of the lethal mutants would be of great value in biosynthetic studies, but only rarely is a lethal mutant found whose defect can be corrected by altering the cultural conditions. Consequently, most investigations of chlorophyll mutants in higher plants have utilized nonlethal types capable of growth and reproduction when planted in soil in a growth chamber, greenhouse, or in the field. Knowledge of the

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inheritance of such mutants enhances their usefulness in biochemical studies.

Interspecific hybridization within the genus *Melilotus* results in many chlorophyll-deficient hybrids (6), but most of these have not been studied genetically. The only hybrid subjected to detailed genetic analysis arose from the cross of *M. alba* Desr. and *M. dentata* (Waldst. & Kit.) Pers. (7). Chlorophyll-deficient F_1 hybrids were preserved by grafting to normal *M. officinalis* (L.) Lam. plants, and were used as the female parents in backcrosses with *M. alba*. Effects of several nonallelic genes controlling chlorophyll deficiency were noted in progenies of the resulting backcross plants. Three of these genes, identified as ch_1, ch_2, and ch_3, produced chlorophyll-deficient plants when homozygous, as well as in certain heterozygous combinations. The triply heterozygous condition was lethal in the seedling stage. Subsequent work suggested that chlorophyll deficiency in the F_1 hybrid of the *M. alba* × *M. dentata* cross resulted from the interaction between nonallelic genes of the two species (7).

Clarke (2) described two spontaneous chlorophyll-deficient mutants of *M. alba* that were semilethal in the greenhouse, but almost 100% of the mutant plants died under field conditions. Each mutant was inherited as an independent monogenic recessive resulting in the production of pale green seedlings. The two genes were designated pg_1 and pg_2.

A recent report from this laboratory (4) deals with the isolation of several types of mutants in an annual strain of *M. alba*, following seed treatments with ethyl methanesulfonate. Numerous chlorophyll-deficient mutants were detected. Many of the deficiencies were lethal, but some mutant lines could be successfully propagated in growth chambers or the greenhouse. Six of these viable, chlorophyll-deficient mutants were available for study when the present work was initiated. The studies reported were conducted to determine the mode of inheritance of these six mutants and to investigate the possibility of allelism among the genes involved.

MATERIALS AND METHODS

The chlorophyll-deficient lines were derived from the same source and grown under essentially the same conditions as the morphological mutants previously described (3). All parental, F_1, and F_2 plants were grown in growth chambers while the F_2 progenies were grown in the greenhouse. Characteristics of the parental lines are given in Table 1. The system used for color classification was as follows: #1 — albino, lethal; #2 — yellow, may be lethal; #3 — yellow-green; #4 — light green; #5 — dark (normal) green. The letter "v" associated with a color class denotes the presence of a prominent leaf venation which was considerably darker in color, particularly in the early stages of growth, than the leaves of the #3 or #4 mutants in which it appeared. The veined mutant was readily distinguished from all other mutants at all stages of growth in the growth chambers as well as the greenhouse. The five nonveined mutants could be distinguished from each other only during the first 2 to 3 weeks of growth in the growth chamber. All mutants were easily distinguished from the normal control at all stages of growth.

Table 1. Characteristics of six chlorophyll-deficient mutant lines and the normal green control.

Line	Color class	Color	Height at maturity, cm	Seed production
Q839	3	Yellow-green	30-38	Good
Q843	4	Light green	15-25	Poor
Q844	4	Light green	20-30	Good
Q851	4	Light green	30-38	Good
Q856	4	Light green	30-38	Fair
Q858	3v	Yellow-green and veined	30-38	Good
Q525	5	Normal green	35-50	Excellent

The crossing procedure was identical to that previously described (3) except that an attempt was made to obtain seed from all normal × mutant crosses and their reciprocals. For complementation studies, crosses among the six mutants were made in all possible combinations, but no attempt was made to obtain all possible reciprocal crosses.

The F_1 plants obtained from each cross were compared to plants of the parental lines with respect to leaf color and venation, height, general vigor, and seed set. For each mutant, 100 to 200 F_2 seeds, obtained by self-pollination of one to three F_1 plants, were planted to obtain F_2 segregation ratios. Data from F_2 progenies segregating for the same phenotype were pooled. The progenies of several F_2 plants classified as recessive were planted to determine whether the recessive condition bred true, and the progenies of at least 19 F_2 plants of the dominant phenotype also were checked for segregation. In this progeny testing, either 18 or 27 seeds of each dominant F_2 plant were used, depending on whether the F_3 families would be expected to segregate into two or four classes, respectively.

RESULTS AND DISCUSSION

Crosses Between Normal and Mutant Lines

F_1 plants were obtained from 10 of the 12 possible reciprocal combinations of the six mutant lines crossed with normal plants. All F_1 plants were phenotypically similar to the normal parent in leaf coloration, plant height, and vigor 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 from five of the six mutant lines were approximately 3:1 (Table 2), suggesting a monogenic inheritance with normal leaf color completely dominant over each chlorophyll-deficient mutant. Phenotypically mutant F_2 plants from each of these five lines bred true in the F_3 generation. F_2 and some F_3 progenies from the sixth mutant line, Q858, segregated into four distinct classes as follows: dark green with normal veins (#5), light green with normal veins (#4), light green with dark green veins (#4v), and yellow-green with light green veins (#3v). The F_2 ratio observed gave a satisfactory fit to a 9:3:3:1 ratio, indicating that two recessive genes were apparently responsible for the chlorophyll deficiency and venation observed in line Q858. The conclusion is reasonable that plants of color class #4 resulted when one of the gene pairs was in the homozygous recessive condition; #4v plants were produced when the other gene pair

Table 2. Chi-square analyses of F_2 and F_3 segregating populations from crosses of six chlorophyll-deficient mutants to normal plants.

Mutant line	Generation*	No. of families	Distribution among color classes #5	#4v	#4	#3v	#3	Chi-square	P value
Q839	F_2 (5)	3	153				46	0.38	0.50-0.75
	F_3 (5)	14	149				41	1.19	0.25-0.50
Q843	F_2 (5)	3	132		44			0.00	> 0.99
	F_3 (5)	13	118		40			0.01	0.90-0.95
Q844	F_2 (5)	2	141		51			0.25	0.50-0.75
	F_3 (5)	15	149		51			0.03	0.75-0.90
Q851	F_2 (5)	1	81		20			1.46	0.10-0.25
	F_3 (5)	15	167		61			0.37	0.50-0.75
Q856	F_2 (5)	2	76		22			0.34	0.50-0.75
	F_3 (5)	11	103		26			1.61	0.10-0.25
Q858	F_2 (5)	1	57	20	22	8		0.61†	0.75-0.90
	F_3 (5)	7	85		25			0.30	0.50-0.75
	F_3 (5)	2	24	8				0.00	> 0.99
	F_3 (5)	12	123	40	29	10		3.52†	0.25-0.50
	F_3 (4v)	15		138		49		0.14	0.50-0.75
	F_3 (4)	8			51	18		0.04	0.75-0.90

* The numbers in parentheses refer to the color class of the plant in the previous generation from which the seed for these plantings was derived. † Tested for goodness-of-fit to a 9:3:3:1 ratio; all others tested against a 3:1 ratio.

Table 3. Chi-square analysis of distributions of segregating and nonsegregating F_3 families from dominant F_2 plants resulting from crosses of six chlorophyll-deficient mutant lines to normal plants.

Mutant line	Color of F_2 plant	F_3 families observed		χ^2 value	P value
		Segregating*	Nonsegregating		
Q839	5	14	6	0.10	.75-.90
Q843	5	13	7	0.03	.75-.90
Q844	5	15	5	0.63	.25-.50
Q851	5	15	5	0.63	.25-.50
Q856	5	11	9	1.23	.25-.50
Q858	5	12:7:2	4	3.26†	.25-.50
Q858	4v	15	4	1.29	.25-.50
Q858	4	8	9	2.94	.05-.10

* Refer to Table 2 for segregation patterns. † Tested for goodness-of-fit to a 4:2:2:1 ratio; all others tested against a 2:1 ratio.

was homozygous recessive, and the #3v plants were caused by a combination of both recessives. Of five F_3 families from #3v F_2 plants, all bred true for the #3v character. All chi-square values for the ratios from individual families, for pooled data, and for heterogeniety were nonsignificant at the 5% level of probability.

For each case in which the segregating F_3 families displayed a ratio of 3 dominant plants:1 recessive plant (Table 2), the numbers of segregating and nonsegregating F_3 families from dominant F_2 plants provided a satisfactory fit to the 2:1 ratio expected in monogenic inheritance (Table 3). Similarly, the pattern of segregating and nonsegregating progenies from normal (color #5) F_2 plants from the Q858 cross provided a satisfactory fit to the 4:2:2:1 ratio expected in dihybrid inheritance.

In light green, veined (#4v) seedlings, the leaves gradually darkened as the plant developed until a normal green color was attained at maturity. The darkened veins were still detectable in mature plants but they were considerably less noticeable than in young seedlings. Leaves of yellow-green, veined (#3v) plants also became slightly darker at maturity with less distinct veins.

All available evidence supports the conclusion that each of the respective chlorophyll deficiencies in the mutants designated as Q839, Q843, Q844, Q851, and Q856 is the result of a monogenic alteration, and that in each case the mutant phenotype is recessive to the dark green color of normal plants. Two independent recessive alleles appear to be responsible for the phenotype of mutant Q858. One of the genes has a phenotypic effect similar to the five single-gene mutants. The other (the veined gene) conditions a chlorophyll deficiency in which the color of the leaf veins is relatively unchanged. Childers and McLennan (1) reported a single-gene mutant in *Medicago sativa* L. that was phenotypically similar to, but more extreme than, the veined mutant observed in line Q858. The *Medicago* mutant had white or yellow-green, veined leaves.

Complementation Studies

F_1 plants from 12 of the 15 crosses made in a diallel involving the six chlorophyll-deficient mutant lines were normal green in color; the three remaining crosses produced only chlorophyll-deficient hybrids (Table 4). Complementation was demonstrated in all crosses involving Q839, Q844, Q856, and the veined gene in Q858, indicating that the four mutant genes, designated ch_4, ch_6, ch_7, and ch_v, respectively, were nonallelic to each other and to the other genes studied. Noncomplementation in the crosses involving Q843, Q851, and the nonveined chlorophyll-deficient gene in Q858 suggested that these mutant genes were allelic. The single locus involved was designated ch_5. Limited F_2 data obtained from these crosses generally supported the interpretation presented above; normal:mutant ratios of 3:1, 9:7, or 27:37 were observed when 1, 2, or 3 pairs of genes, respectively, were segregating. No information was obtained in the current study concerning possible relationships between the five mutant genes and the chlorophyll-deficient mutants described previously (2, 7).

Table 4. Phenotypes of F_1 plants obtained from intercrosses among six chlorophyll-deficient mutants of annual *M. alba*.

Mutants crossed	No. F_1 plants	Color class		Mutant genotypes
		Parents	F_1	
Q839 × Q843	3	3 × 4	5	$ch_4ch_4 \times ch_5ch_5$
Q844 × Q839	3	4 × 3	5	$ch_6ch_6 \times ch_4ch_4$
Q839 × Q851	3	3 × 4	5	$ch_4ch_4 \times ch_5ch_5$
Q856 × Q839	5	4 × 3	5	$ch_7ch_7 \times ch_4ch_4$
Q839 × Q858	9	3 × 3v	5	$ch_4ch_4 \times ch_5ch_5ch_vch_v$
Q844 × Q843	2	4 × 4	5	$ch_6ch_6 \times ch_5ch_5$
Q851 × Q843	14	4 × 4	4	$ch_5ch_5 \times ch_5ch_5$
Q843 × Q856	7	4 × 4	5	$ch_5ch_5 \times ch_7ch_7$
Q843 × Q858	4	4 × 3v	4	$ch_5ch_5 \times ch_5ch_5ch_vch_v$
Q844 × Q851	3	4 × 4	5	$ch_6ch_6 \times ch_5ch_5$
Q856 × Q844	2	4 × 4	5	$ch_7ch_7 \times ch_6ch_6$
Q844 × Q858	6	4 × 3v	5	$ch_6ch_6 \times ch_5ch_5ch_vch_v$
Q851 × Q856	1	4 × 4	5	$ch_5ch_5 \times ch_7ch_7$
Q858 × Q851	1	3v × 4	4	$ch_5ch_5ch_vch_v \times ch_5ch_5$
Q856 × Q858	3	4 × 3v	5	$ch_7ch_7 \times ch_5ch_5ch_vch_v$

Thus far, detailed studies of the effects of the various mutant genes on the photosynthetic apparatus have not been made. In a recent review, Nelson (5) emphasized that only a minority of chlorophyll mutants are caused by a block in chlorophyll synthesis. Some chlorophyll-deficient phenotypes are due to blocks in the formation of either accessory pigments or the structural components of the chloroplasts whose normal development is essential for stability of the photosynthetic pigments. Others result from the absence of an essential metabolite which leads to the inability to attain or maintain normal chloroplast structure or chloroplast pigmentation. With regard to the *Melilotus* mutants described in the present paper, preliminary chromatographic evidence suggested that extracts from leaves of Q843, Q851, and Q858 lacked a yellow-green pigment, thought to be chlorophyll *b*, that was present in extracts from the other mutants and from normal plants. It is significant that the three mutants which behaved as alleles in complementation studies all appeared to lack the same green pigment. Much additional work is needed on the biochemistry of these chlorophyll-deficient mutants.

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