

GENETICS OF CHLOROPHYLL DEFICIENCIES IN  
RED CLOVER (*TRIFOLIUM PRATENSE* L.)

I. ALBINOS.

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CONTENTS

	PAGE
I. Introduction . . . . .	441
II. Previous investigations . . . . .	442
III. Material and methods . . . . .	443
IV. Tests of non-identity of white and yellow factors . . . . .	444
V. w1 w1 genotype . . . . .	446
VI. w2 w2 genotype . . . . .	447
VII. w3 w3 genotype . . . . .	448
VIII. w4 w4 genotype . . . . .	449
(1) Pre-germination lethal effect of w4 . . . . .	450
(2) Linkage tests with w4 . . . . .	451
IX. w5 w5 genotype . . . . .	452
X. w6 w6 genotype . . . . .	452
XI. w7 w7 genotype . . . . .	453
XII. w8 w8 genotype . . . . .	454
XIII. Other white lethal seedling types . . . . .	455
XIV. White seedlings giving aberrant ratios . . . . .	455
XV. Summary and conclusions . . . . .	456
References . . . . .	457

I. INTRODUCTION

SINCE red clover breeding was started at the Welsh Plant Breeding Station about eighteen years ago, many chlorophyll deficiencies have been isolated. Most of these have appeared by chance in inbred cultures during the course of practical breeding and genetical investigations, but several types have also been discovered as a result of systematic search among commercial varieties. These chlorophyll-deficient mutants often exhibit very striking differences in chlorophyll intensities and survival values. They have been classified, according to the amount of chlorophyll they contain and their viability, into seven main groups, namely, (1) lethal albinos, (2) lethal and sublethal yellow types, (3) lethal and sublethal yellow-green types, (4) lethal and sublethal

light green types, (5) surviving yellow-green types, (6) surviving light green types and (7) surviving variegated types. Each of the surviving groups has been further subdivided on the basis suggested by Demerec (1925) into (a) vital types which have enough chlorophyll for photosynthesis, but which remain pale to yellow-green through all stages of development; (b) progressive types which are deficient in chlorophyll in the seedling stage, but become green later; and (c) digressive types in which the chlorophyll intensity decreases with age. Though only approximate, since there is no clear-cut distinction between the various groups and subgroups, this classification has justified itself as a means of bringing together, for the purposes of recording and study, factors which are similar, or fairly similar, in their general expressions.

A considerable amount of data on the mode of inheritance, and on the genetic and linkage relations of these various chlorophyll deficiencies has been accumulated, but in the interest of space only the data on white seedling characters will be dealt with in this paper. The results of the investigations on various other chlorophyll-deficient types will be published shortly in a series of papers.

## II. PREVIOUS INVESTIGATIONS

Chlorophyll deficiencies have been observed in a large number of species. They have been found to be particularly numerous in naturally cross-pollinated plants, such as maize and red clover, but are also known to occur occasionally in exclusively self-fertilized crops. As a result of the intensive researches of Emerson (1912), Lindstrom (1921), Demerec (1924), Carver (1927), Jenkins & Bell (1930), and other workers, our knowledge of the genetics of the chlorophyll characters in maize is far more comprehensive than in other crops. Hutchison (1922) reported that twenty-four pairs of factors responsible for chlorophyll development in maize were then known. Several new chlorophyll factors have been isolated in this crop since this report was published. Chlorophyll-deficient characters have also been intensively studied in sorghum (Karper & Conner, 1931), in barley (Hallquist, 1926; Collins, 1927; and Imai, 1935), in wheat (Smith & Harrington, 1929), in rice (Ramiah & Ramanujan, 1935), in perennial rye-grass (Jenkin, 1928), in *Vicia faba* (Darlington, 1929), and in several other cultivated crops. A comprehensive survey of literature on chlorophyll deficiencies is given by De Haan (1933).

The only previously published data on the inheritance of chlorophyll abnormalities in red clover are those recorded by Wexelsen (1932) and

Nijdam (1932). Wexelsen found two chlorophyll-deficient types, one with pure yellow seedlings and the other with white mosaic cotyledons, which he regarded as being due to duplicate factors. This conclusion was based, in the case of yellow seedling character, on data for five families which gave a total of seventy-two green and eleven yellow seedlings, and, in the case of the type with white mosaic cotyledon leaves, on counts obtained in two progenies which gave sixty-seven green and seven white mosaic seedlings. It is interesting to note in this connexion that out of the large number of characters which I have investigated I have not found a single case which remotely suggests the occurrence of duplicate factors in red clover. In view of the inadequacy of the data it is doubtful whether Wexelsen was justified in drawing such a conclusion. In addition to the two types already referred to, Wexelsen found a type with yellow-green cotyledons, which later became green; this gave an  $F_2$  distribution of fifty-three green and thirty-one yellow-green seedlings.

Nijdam found in red clover a light green surviving type which was due to a single recessive factor, an aurea-tinted plant, the genetic behaviour of which was not reported, and a variegated type which was maternally inherited. I may state in this connexion that I have not yet observed a single case of maternal inheritance in red clover.

### III. MATERIAL AND METHODS

Various types of chlorophyll deficiencies have been found in Montgomery, Cornish Marl, English late, English broad red, Vale of Clwyd, American medium, American Mammoth and Swedish late; in fact, in all varieties subjected to inbreeding at Aberystwyth. Since Montgomery has been more extensively used for practical breeding than any other form, most of the types were derived from this variety, but it is known that they occur in nearly the same frequency in the English late variety, and from the rather limited data available it seems probable that they are equally numerous in other varieties.

With the exception of y3, which was carried by a self-fertile plant ( $S^1S^x$ ), all the chlorophyll-defective lethals reported in this and subsequent papers were derived from completely self-sterile parents. Segregating  $F_2$  and  $F_3$  progenies were usually obtained by intercrossing heterozygous sister plants or by mating the heterozygous parents with their heterozygous offspring; occasionally half-sisters and other related plants were intercrossed. As previously stated, most of the chlorophyll-deficient types originally appeared by chance in inbred cultures. When a new type appeared, the procedure usually adopted was to repeat the

cross which gave rise to it, and at the same time one or both of the heterozygous  $F_1$  parent plants were out-crossed to unrelated individuals. A number of the  $F_1$  plants from these new crosses were then crossed either chainwise or diallely with each other, or back-crossed directly to the heterozygous parents. From the standpoint of securing the largest possible number of segregating progenies, the latter method has an advantage over the former, in that one out of every two parent  $\times$  sib crosses would be expected to segregate as compared with only one out of four sib  $\times$  sib crosses.

With the exception of the sublethal  $w8 w8$  genotype, few of which survived to maturity, all the albinos reported here were lethal in the early seedling stage, and on that account the recessives, apart from  $w8 w8$  plants, could not be used for back-crossing.

The cross-pollinations were made by hand in bee-protected greenhouses. The seeds were sown in heated greenhouses during the following winter. They were rubbed between sheets of sandpaper so as to allow water to permeate through the testae, and sown in boxes at about 1 in. apart and covered lightly with about  $\frac{1}{4}$  in. of soil; even the weakest seedlings experienced very little difficulty in breaking through this shallow covering.

Seeds from reciprocal crosses were invariably sown separately, and in most instances the small under-developed shrivelled seeds when present in appreciable numbers were also sown apart from well-developed seeds.

The seedlings were kept under observation from the time they germinated until all the recessives had died. Those belonging to the different chlorophyll classes were marked as they appeared through the soil by means of different coloured pegs.

Ridgway's *Color Standards* (1912) was exclusively used for determining the various tints and shades of colour of the different types. Owing to the limited range of the tints and shades of yellows and greens represented in *Color Standards*, this method of designating the seedling colours was not entirely satisfactory, since it was seldom possible to obtain a good match.

#### IV. TESTS OF NON-IDENTITY OF WHITE AND YELLOW FACTORS

Eight white lethal, six yellow lethal and one yellow sublethal type, derived from apparently quite unrelated stocks, have been investigated. To determine whether these fifteen types were in fact due to different

factors, plants heterozygous for each type were intercrossed with each other in nearly all possible combinations. These crosses gave, on the average, about fifty seedlings; those which gave less than ten seedlings were in all cases repeated. The observations on these  $F_1$  seedlings are summarized in Table I.

$F_1$  progenies resulting from crosses between plants heterozygous for simple recessive chlorophyll-deficient factors would segregate, if only one recessive factor was involved, for green and chlorophyll seedlings in the ratio of 3 : 1, but if two different recessive factors were involved only green seedlings would be obtained. As may be seen from Table I, every one of the ninety-two crosses between plants heterozygous for one or

TABLE I

*Diallel crosses between plants heterozygous for eight white and seven yellow lethal factors (the sign "N" indicates that the  $F_1$  seedlings were all normal green)*

	W1	W2	W3	W4	W5	W6	W7	W8	Y1	Y2	Y3	Y4	Y5	Y6	Y7
W1	—	N	N	.	.	.	N	.	N	N	N	.	.	N	N
W2	N	—	N	N	N	.	N	N	N	N	N	.	.	N	N
W3	N	N	—	N	N	.	N	N	N	N	N	.	.	N	N
W4	.	N	N	—	N	.	N	N	N	N	N	N	.	N	N
W5	.	N	N	N	—	N	N	N	N	N	N	N	N	N	N
W6	.	N	.	.	N	—	N	N	N	.	.	N	.	.	N
W7	N	N	N	N	N	N	—	N	N	N	N	N	N	N	N
W8	.	N	N	N	N	N	N	—	N	N	N	N	N	N	N
Y1	N	N	N	N	N	N	N	N	—	N	N	N	N	N	N
Y2	N	N	N	N	N	.	N	N	N	—	N	N	N	N	N
Y3	N	N	N	N	N	.	N	N	N	N	—	N	N	N	N
Y4	.	N	.	N	N	N	N	N	N	N	N	—	N	N	N
Y5	.	N	N	N	N	.	N	N	N	N	N	N	—	N	N
Y6	N	N	N	N	N	.	N	N	N	N	N	N	N	—	N
Y7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	—

other of the fifteen white and yellow seedling factors gave only green seedlings. Unfortunately the W1 w1 and W6 w6 plants died before all the crosses with them could be made; but even if these two are excluded the other crosses are sufficiently complete to indicate that thirteen out of the fifteen factors tested were genetically different. The w2 w2 genotype has appeared in three unrelated stocks, but so far all the other fourteen genotypes have appeared only once.

It is realized that these tests would have been more conclusive if it had been possible to obtain  $F_2$  dihybrid segregations from each of the ninety-two  $F_1$  crosses. This would have entailed an enormous amount of work, particularly since only about 7%, or, more precisely, one out of sixteen, of the  $F_2$  progenies would be expected to give dihybrid segregations.

*White lethal seedlings w1 w1 to w8 w8 genotypes*

None of these eight types was pure albino in the true sense of the word, since they all contained on emergence a very small but variable amount of yellow pigment which gave the cotyledons a faint yellowish or creamy tint; but within a few days the yellow pigment was bleached to pure white.

V. **w1 w1** GENOTYPE

This type appeared in an  $F_2$  progeny of a Montgomery  $\times$  Cornish Marl cross. It is completely lacking in chlorophyll; at first the cotyledons, which are of a normal size on emergence, are faintly tinged with yellow (Ridgway XXX 23' e), but fade to pure white within 4-6 days. The seedlings are lethal in the cotyledon stage.

Five  $F_2$  progenies segregated for w1 w1 type; the data on these are given in Table II.

TABLE II  
 $F_2$  segregations for w1 w1 seedlings

Pedigree no.	Observed			Deviation (from 3 : 1)
	w1	w1	Total	
637(2)4 $\times$ 5	233	132	370	+39.5 $\pm$ 8.33*
1315(2)2 $\times$ 4	105	19	124	-12.0 $\pm$ 4.82
1108(2)1 $\times$ 2	55	11	66	-5.5 $\pm$ 3.52
1108(2)1 $\times$ 7	74	20	94	-3.5 $\pm$ 4.20
2008(1)	88	27	115	-1.75 $\pm$ 4.64

\* Standard errors (and not probable errors) are used throughout this paper.

In three  $F_2$  progenies, namely, 1108(2)1  $\times$  2, 1108(2)1  $\times$  7 and 2008(1), the observed distribution of green and white seedlings agrees fairly closely with expectation on the basis of a monohybrid segregation, the deviation  $\div$  s.e. in one progeny was about 1.6, and in the other two less than 1.0. The other two progenies gave results which deviated greatly from the calculated 3 : 1 ratios—one giving a very significant excess, and the other a significant deficiency of white seedlings; the odds are very great against the observed deviations being due merely to chance, since an excess of the magnitude of 39.5  $\pm$  8.33 given by 637(2)4  $\times$  5 family would be expected to occur as a result of chance fluctuation only once in about 500,000 trials, and a deficiency of the order of 12.0  $\pm$  4.82 obtained from 1315(2)2  $\times$  4 would occur only once in about 100 trials. That the deviation given by 637(2)4  $\times$  5 progeny is genetically significant receives further support from the fact that the reciprocal and the repeat matings gave distributions similar to that obtained from the original

cross. The original 4 ♀ × 5 ♂ cross gave 68 green and 38 white seedlings, the repeat cross 99 green and 49 white seedlings, and the reciprocal 5 ♀ × 4 ♂ mating 71 green and 45 white seedlings; the excesses in the recessive class being  $11.5 \pm 3.0$ ,  $16.0 \pm 3.1$  and  $12.0 \pm 3.6$  respectively.

The widely divergent ratios obtained from these five progenies may be explained by assuming that *w1* is loosely linked with the sterility allelomorphs (**S**). The facts fit this hypothesis very well if it is assumed: (1) that the  $F_1$  parent plants of each of the three progenies which gave normal 3 : 1 distributions were carrying four different sterility allelomorphs; these three crosses may be represented thus (**S**<sup>1</sup>**W1**) (**S**<sup>2</sup>*w1*) × (**S**<sup>3</sup>**W1**) (**S**<sup>4</sup>*w1*); (2) that the two  $F_1$  parent plants of the  $F_2$  progeny, 637(2)4 × 5, which gave a significant excess in the recessive class, had a common **S** factor linked with **W1**; the constitution of the two parents being (**S**<sup>1</sup>**W1**) (**S**<sup>2</sup>*w1*) × (**S**<sup>1</sup>**W1**) (**S**<sup>3</sup>*w1*) with about 28.6% crossing-over between **S** and **W1**; and (3) that the immediate parents of the 1315(2)2 × 4 which gave a marked deficiency of white seedlings also had a common **S** factor, but that this was linked with *w1*; their factorial compositions may, therefore, be represented as (**S**<sup>4</sup>**W1**) (**S**<sup>2</sup>*w1*) × (**S**<sup>3</sup>**W1**) (**S**<sup>2</sup>*w1*) with a computed crossing-over of about 30.7% between **S** and **W1**.

These observations indicate that this white seedling character is due to a simple recessive factor which has been designated *w1*. Also it may be tentatively assumed that *w1* factor is located in the **S-C** linkage group.

#### VI. *w2 w2* GENOTYPE

This white lethal genotype is of triple origin. It has appeared on separate occasions in inbred progenies of three Montgomery crosses, and, as far as is known, the parent plants of these crosses were quite unrelated. None of the other white and yellow chlorophyll-deficient types so far investigated has appeared more than once.

The *w2 w2* genotype is phenotypically indistinguishable from *w1 w1*, *w3 w3*, *w5 w5*, and *w6 w6* genotypes.

This type is entirely lacking in chlorophyll. The cotyledons which at first are tinged with yellow soon fade to pure white. The seedlings perished in the cotyledon stage in about 25-30 days.

Ten  $F_2$  progenies derived from five  $F_1$  crosses segregated for *w2 w2* seedlings. The results obtained are summarized in Table III.

The results given by each individual  $F_2$  progeny are in very close agreement with expectation, assuming a single factor difference between the green and white seedlings. The total observed distributions for eleven

448 *Genetics of Chlorophyll Deficiencies in Red Clover*

progenies were 668 green and 234 white seedlings, the deviation from a 3 : 1 ratio being only  $8.5 \pm 13.00$ . It is clear, therefore, from these results that this white seedling character is determined by a simple recessive factor. This has been given the symbol *w2*. As may be seen from Table I, the *w2* factor has been shown to be genetically different from the other fourteen chlorophyll-deficient lethal factors.

TABLE III  
*F<sub>2</sub> segregations for w2 w2 genotypes*

Pedigree no.	No. of <i>F<sub>2</sub></i> progenies	Observed			Deviation (from 3 : 1)
		<i>W2</i>	<i>w2</i>	Total	
483	2	86	32	118	+2.5 ±4.70
485	3	190	56	246	-4.5 ±6.79
476	3	115	47	162	+6.5 ±5.51
1681	1	121	41	162	+0.5 ±5.51
1790	1	44	15	59	+0.25 ±3.33
2009	1	112	43	155	+4.25 ±5.39
Total: Observed		668	234	902	
Calculated (3 : 1)		676.5	225.5	902	+8.5 ±13.00

VII. *w3 w3* GENOTYPE

This type is another pale yellow albino which originated from a Montgomery cross. Except that the cotyledons are slightly smaller and weaker it is indistinguishable from *w1 w1* and *w2 w2* genotypes. So far only one *F<sub>2</sub>* progeny has segregated for the *w3* albinos, and this gave 136 green and 27 white seedlings, a deficiency in the recessive class of  $13.75 \pm 5.53$  seedlings from the expected 3 : 1 ratio. Since the deviation is 2.5 times its standard error it seems probable that the shortage of white seedlings is statistically significant. It is possible that this deficiency was partly or wholly due to pre-germination elimination of *w3 w3* genotypes, as was found to be the case in the *w4 w4* type.

It is realized that the data obtained so far are too inadequate to allow definite conclusions to be drawn, but they seem to suggest that this white seedling character is due to the action of a simple recessive factor.

Crosses between individuals heterozygous for *w3* and plants heterozygous for six white seedling factors *w1*, *w2*, *w4*, *w5*, *w7* and *w8*, and also plants heterozygous for six yellow seedling factors *y1*, *y2*, *y3*, *y5*, *y6* and *y7* produce green seedlings only. It may, therefore, be safely assumed that *w3* is genetically different from at least twelve out of the fourteen lethal factors investigated. It has not yet been tested with *w6* and *y4*. The striking dissimilarities of their phenotypic expressions

strongly suggest that w3 and y4 are different factors. The w3 w3 and w6 w6 genotypes are very similar but are of different origin, the former being derived from Montgomery variety and the latter from Vale of Clwyd variety.

## VIII. w4 w4 GENOTYPE

This chlorophyll mutant originated in  $F_2$  progenies of a Montgomery cross. The cotyledon leaves are at first pale yellow in colour, but soon fade to pure white. The seedlings die in about 20-30 days after germination. This is the weakest chlorophyll-deficient type yet found. Its cotyledon leaves are very small; even at germination they are only about one-third to one-half the size of those of their green sibs. Though the seeds appear to germinate quite normally after being rubbed, the seedlings are so feeble that they have considerable difficulty in bursting their seed coats, with the result that the cotyledons carried on elongated hypocotyls are often firmly encased in the testae during the whole of the brief life cycle of the seedlings. Data on nine progenies segregating for this type are given in Table IV.

TABLE IV  
*F<sub>2</sub> segregations for w4 w4 seedlings*

Pedigree no.	Observed			Deviation (from 3 : 1)
	W4	w4	Total	
557(2)25 × 93	1098	107	1205	-194.25 ± 15.03
557(2)1 × 25	51	9	60	- 6.0 ± 3.35
2100(2)2 (selfed)	897	18	915	-210.75 ± 13.09
2100(2)7 ♀ × 2	387	2	389	- 95.25 ± 8.54
2263(2)1 × 4	585	7	592	-141.0 ± 10.53
4033(1)	737	35	772	-158.0 ± 12.03
4034(1)	504	15	519	-114.75 ± 9.86
4036(1)	960	32	992	-216.0 ± 13.65
4037(1)	782	14	796	-185.0 ± 12.21
Total: Observed	6001	239	6240	
Calculated (3 : 1)	4680	1560	6240	-1321 ± 34.26

$\chi^2=1527.34.$   $P$ =very small.

The nine progenies which segregated for w4 w4 gave very marked deficiencies in the recessive class on the basis of 3 : 1 ratios; in the case of eight of these progenies the deviations from expectation range from 11.1 to 15.8 times their standard errors, and the odds against these differences being due merely to chance are inconceivably great. The other family, 557(2)1 × 25, also gave a shortage of white seedlings, but the deviation—6.0 ± 3.35—is such as might possibly have occurred as a result of chance fluctuations.

The differences from expectation calculated on the assumption that

the white seedlings are due to the action of duplicate or triplicate factors are also very great. For 15 : 1 distributions,  $\chi^2$  is 782.5, and  $P$  is very small, and for 63 : 1 ratios,  $\chi^2$  is 501.3, and  $P$  is still very small.

A pronounced shortage in the recessive class would be expected in some of the  $F_2$  crosses if  $w_4$  was located in the **S-C** linkage group, and was closely linked with sterility allelomorphs, but for all nine progenies to give deficiencies of white seedlings it is necessary to assume not only that  $w_4$  and **S** are closely linked, but also that the  $F_1$  parents of each progeny had a common **S** factor coupled with  $w_4$  factor. Since the parent plants of these crosses have been extensively used in connexion with the study of the linkage intensity of **S** and  $c_p$ , a simple recessive pink flower-colour factor, their constitutions in respect of **S** and  $w_4$  are known. The factorial compositions of immediate parents of 4033(1) and 4034(1) progenies were  $S^1W_4.S^2w_4 \times S^3W_4.S^2w_4$ , and of 2100(2)7  $\times$  2, 4036(1) and 4037(1) progenies were  $S^3W_4.S^2w_4 \text{ } \text{♀} \times S^1W_4.S^2w_4 \text{ } \text{♂}$ . Assuming close linkage between **S** and  $w_4$ , these five progenies would be expected to give marked deficiencies of white seedlings. The constitutions of the  $F_1$  parents of 557(2)25  $\times$  93 and 2263(2)1  $\times$  4 progenies are known to be  $S^1W_4.S^2w_4 \times S^1W_4.S^3w_4$ , in which  $W_4$  is associated with a common **S** factor. If these two genes are strongly linked, a marked excess of the recessive seedlings, instead of a great shortage actually obtained, would be expected from these two crosses. It is evident from the constitutions of these two progenies that **S** and  $w_4$  are not linked, and therefore the sterility allelomorphs acting as gamete lethals are not responsible for aberrant ratios given by  $w_4$ .

(1) *Pre-germination lethal effect of  $w_4$* . It was observed that some of these crosses contained a rather unusually large number of under-developed seeds. The seeds of five crosses were separated arbitrarily into two classes, and sown separately; one class consisted of plump and well-developed seeds and the other of small, shrivelled and under-developed seeds. The number of green and  $w_4 w_4$  seedlings which germinated in each class are given in Table V.

As may be seen from Table V the "good" and "poor" seeds from the same crosses gave widely divergent distributions of green and white seedlings. In every case a great and significant shortage of recessive seedlings on the basis of 3 : 1 ratio was obtained from the well-developed seeds, while a significant excess of  $w_4 w_4$  seedlings was obtained from the under-developed seeds in four out of five crosses tested. Taking the total data on the five progenies, the "good" seeds gave only 2.3% white seedlings, while the "poor" seeds gave as many as 40.7%.

As might be expected, the germination of the under-developed seeds was very poor. The seeds of the 557(3)1 × 25 family were germinated in the laboratory, and the comparatively high germination results given by the poor seeds of this family are accounted for by the fact that testae of the viable seeds which proved too weak to germinate without assistance were carefully removed by hand.

In addition to the small shrivelled seeds that were sown, all the crosses had an appreciable proportion of very small, badly shrivelled and obviously non-viable seeds which were not sown. Since the viable "poor" seeds gave a marked excess of white seedlings, it seems highly probable that a large proportion of the non-viable seeds were also of w4 w4 type. There appears to be no doubt from these observations that the normal development of seeds is greatly impeded when w4 is present

TABLE V

*Number of green and w4 w4 seedlings from "good" and "poor" seeds of five F<sub>2</sub> progenies*

Pedigree no.	Good seeds					Poor seeds				
	Seeds sown	Seedlings				Seeds sown	Seedlings			
		W4	w4	Total	% w4		W4	w4	Total	% w4
557(2)25 × 93	683	584	23	607	3.8	122	50	43	93	46.3
557(2)1 × 25	39	38	1	39	2.6	30	13	8	21	38.1
4033(1)	432	400	14	414	3.4	37	4	3	7	42.9
4034(1)	512	491	5	496	1.1	61	13	10	23	43.5
4037	845	773	10	783	1.3	146	19	4	23	17.4
Total	2511	2286	53	2339	2.3	396	99	68	167	40.7

in the duplex condition. This white seedling character seems to be due to a simple factor which, when present in the recessive condition, acts not only as a chlorophyll-deficient early zygotic lethal but also partly as a pre-germination lethal, a fact which accounts for deficiency of the recessive seedlings.

w4 has been shown to be genetically different from all the other white and yellow factors considered here with the exception of w1 and w6 genes with which it has not been tested. The fact that w1 and w6 do not act as pre-germination lethals strongly suggests that they are different from w4.

(2) *Linkage tests with w4.* It has already been demonstrated that w4 and S (sterility) are inherited independently. It is also known that w4 is not linked with c, a white flower colour, and since c is linked with S with about 35% crossing-over it is evident that w4 is not located in the S-C linkage group.

## 452 *Genetics of Chlorophyll Deficiencies in Red Clover*

The only other factor with which it has so far been tested is *m*, a simple recessive gene for absence of leaf-markings, and that in only one  $F_2$  progeny, viz. 2263(2)7  $\times$  2. The constitutions of the  $F_1$  plants which gave rise to the  $F_2$  were MW4.mw4  $\times$  MW4.mw4. Since the w4 w4 seedlings were lethals, only the green seedlings could be scored for leaf-markings, and these consisted of 376 plants with leaf-markings and 76 plants without. The deficiency in the no leaf-marking class of  $37 \pm 9.21$  from expectation on the basis of free assortment of *m* and w4 is highly significant, and suggests a rather weak linkage between *m* and w4 with about 38.85% crossing-over.

### IX. w5 w5 GENOTYPE

This white seedling type which recently appeared in an  $F_2$  Montgomery family is phenotypically indistinguishable from w1 w1, w2 w2, w3 w3 and w6 w6 seedlings. As in the case of all the white seedling types, the cotyledon leaves of w5 w5 genotype are smaller than those of their green sibs. The faint yellow tinge with which they are pigmented at germination soon bleaches to pure white without a trace of green, and all died about 25-30 days before the plumules appeared.

The only data at present available on this type are those obtained from a small  $F_2$  family which gave 64 green and 18 white seedlings. This is clearly a 3 : 1 ratio, the deviation being only  $2.5 \pm 3.92$ . This result, although too inadequate to permit of a definite conclusion being drawn, strongly suggests that there is only a single factor difference between the normal green and white seedlings.

Reference to Table I will show that the w5 factor responsible for this type is genetically different from the six white seedling genes and the seven yellow seedling genes with which it was tested.

### X. w6 w6 GENOTYPE

This is another new pure white seedling type which was recently discovered in an  $F_1 \times F_2$  progeny derived from a Vale of Clwyd cross. As already stated, it is very similar in appearance to w1, w2, w3 and w5 types.

So far only one family has segregated for this type, and this gave 210 green and 58 white seedlings. The observed results are in fairly close agreement with expectation on the assumption that w6 w6 seedlings were the expression of a simple recessive factor, since the difference is only  $9 \pm 7.08$ . w6 has not been tested in regard to its genetic relations with all the other fourteen w and y seedling factors. It has, however,

been intercrossed with  $w_2$ ,  $w_5$ ,  $w_7$ ,  $w_8$ ,  $y_1$ ,  $y_4$  and  $y_7$ , and since the resulting  $F_1$  seedlings were all green, we are justified in assuming that  $w_6$  is different from these seven factors. It is highly probable, from the differences in their phenotypical expression, that  $w_6$  is also genetically different from  $y_2$ ,  $y_3$ ,  $y_5$  and  $y_6$ . Since there is no evidence to the contrary at present available, we must admit the possibility that  $w_6$  may be genetically the same as one of the three white factors,  $w_1$ ,  $w_3$  or  $w_4$ , with which it has not been tested.

#### XI. $w_7 w_7$ GENOTYPE

This type, which is also of Montgomery origin, is characterized by irregular small green patches on the cotyledon leaves. The amount of chlorophyll present is exceedingly variable; in some seedlings it is only just visible to the naked eye as small specks, but even in the most intensely chlorophyll-pigmented individuals the total area covered by the green patches, which is mainly confined to the lower half of the leaves and the upper parts of the petioles, seldom exceeds 25% of the leaf surface. The ground colour of the cotyledons is at first a pale yellow colour, but this fades in a few days to pure white. All seedlings die before they reach the second trifoliate leaf stage; those with mere traces of chlorophyll usually perish in the cotyledon stage, while those with greater chlorophyll intensities generally survive to the simple and first trifoliate leaf stages. In these seedlings the simple and first trifoliate leaves are very small and sessile with green patches on white ground, the pigmented area being mainly confined to the middle of the lamina.

So far only two  $F_2$  progenies have segregated for  $w_7 w_7$  seedlings. The data obtained were:

Pedigree no.	Observed			Deviation (from 3 : 1)
	$w_7$	$w_7$	Total	
464	267	84	351	-3.75 $\pm$ 8.11
2643	132	38	170	-4.5 $\pm$ 5.65
Total	399	122	521	-8.25 $\pm$ 9.88

In both progenies the observed numbers of green and white-green seedlings were in very close agreement with expectation on the basis of monofactorial segregation; in the case of 464 progeny the difference from expectancy was only 3.75  $\pm$  8.11, and, in the case of 2643 progeny, only 4.5  $\pm$  5.65. It is evident, therefore, that  $w_7 w_7$  seedlings are due to the action of a simple recessive gene. As may be seen from Table I, this factor was found to be genetically different from all the other fourteen white and yellow seedling factors investigated.

XII. *w8 w8* GENOTYPE

This genotype was first isolated in an  $F_2$  progeny of a Montgomery cross. It is exceedingly variable in its expression, more particularly in the amount of chlorophyll carried in the cotyledon leaves. Some of the seedlings appeared to be entirely lacking in chlorophyll, while others had small but variable amounts of chlorophyll in the form of diffused green patches on white ground, and were very similar in appearance to *w7 w7* seedlings. The pure white seedlings, and those with low chlorophyll content, were lethal in the cotyledon stage, but most of the patchy green-white seedlings survived to produce very small sessile simple first and second trifoliate leaves, and occasionally the third leaf, before they perished. With the exception of narrow green areas along the mid-ribs, the first-formed leaves were pure white.

Out of a total of 202  $F_2$  *w8 w8* seedlings, only five survived beyond the fourth leaf stage, and of these, three reached maturity despite their extremely slow rate of development during the early- and mid-seedling stages. These became progressively more green and from late seedling stage onwards, though much smaller and weaker, they were almost indistinguishable in colour from their normal green sibs.

In the following spring the variegated seedling character reappeared on the young leaves as green mottling on white ground, but by the beginning of May the recessive plants were again full green. Two of these recessive plants were used for back-crossings. The data obtained on these back-crosses, as well as the  $F_2$  results, are given in Table VI.

TABLE VI  
*F<sub>2</sub> and back-cross data on w8 w8 genotype*

Pedigree no.	Observed			Deviation
	<i>w8</i>	<i>w8</i>	Total	
<i>F<sub>2</sub></i> data:				
468(2)4 × 7	306	82	388	- 15.0 ± 8.53
468(2)47 × 55	192	91	283	+ 20.25 ± 8.47
468(2)55 × 60	83	29	112	+ 1.0 ± 4.58
Total: Observed	581	202	783	
Calculated (3 : 1)	587.25	195.75	783	+ 6.25 ± 12.11
Back-crosses:				
2626(1)	59	62	121	+ 1.5 ± 5.50
2628(1)	93	83	176	- 5.0 ± 6.63
Total: Observed	152	145	297	
Calculated (1 : 1)	148.5	148.5	297	- 3.5 ± 8.62

It will be seen from Table VI that one  $F_2$  family gave an almost significant deficiency of *w8* type, deviation ÷ s.e. being 1.8; another  $F_2$

family gave what is probably a significant excess of recessive seedlings, in this case the deviation  $\pm$  s.e. is 2.4, while the third  $F_2$  family gave nearly an exact 3 : 1 ratio. The totals for the three  $F_2$  progenies were 581 green and 202 white seedlings, an excess in the recessive class of only  $6.25 \pm 12.11$  on the basis of monohybrid segregation.

The results obtained from the two back-crosses conform very closely to the expected 1 : 1 ratios. The total numbers were 152 green and 145 white seedlings, a difference of only  $3.5 \pm 8.62$  from expectancy.

It is evident from these observations that this white-green genotype is determined by a simple recessive factor, w8.

This factor has been found to be genetically different from all the thirteen white and yellow seedling factors with which it has been tested. Its phenotypical expression strongly suggests that w8 is also different from w1, the only factor with which it has not been tested.

#### XIII. OTHER WHITE LETHAL SEEDLING TYPES

In addition to the eight genotypes reported above ten other white lethal types have been isolated in red clover. Nine of these were pure albinos and died in the cotyledon leaf stage, while the other type has white cotyledon leaves slightly tinged with green; some of the seedlings of this form survived until the fourth leaf stage. It would seem from the limited data at present available that nine of these types are inherited on a simple Mendelian basis, but one of the pure albinos gave aberrant  $F_2$  ratios, in one case 242 green and 41 white seedlings, and in the other 235 green and 39 white seedlings in the only two progenies in which it has so far appeared.

Except in a few instances their genetic relations with one another and with w1 to w8 genotypes have not yet been investigated. It is possible, therefore, that some of these types are due to the same factors. They all originated from different, and as far as is known, unrelated parentage, six types being derived from Montgomery, three from English late varieties, one from a Swedish late  $\times$  wild cross and one from a Montgomery  $\times$  Cornish Marl cross.

#### XIV. WHITE SEEDLINGS GIVING ABERRANT RATIOS

Albino seedlings which do not conform to Mendelian ratios are not infrequently found in sib crosses, and distributions have been obtained ranging from about six to 100 green seedlings to one white one. It is known that some of the white seedlings which occasionally appear in crosses between homozygous and heterozygous plants have originated

as a result of pseudo-fertilization of the heterozygous individual. But pseudo-self-fertilization does not account for all the aberrant results obtained, as crosses between entirely self-sterile plants sometimes give a very pronounced shortage of albinos. For instance, in a cross between two completely self-sterile sibs, the numbers of green and white seedlings were 128 : 22, and in the reciprocal cross 107 : 6. The fact that these types were invariably completely devoid of chlorophyll and were perceptibly smaller and weaker than the normal albino types seems to suggest the possibility that they may be due to simple recessive genes acting, in the main, as pre-germination lethals, or that they may be formed as a result of chromosome aberrations.

#### XV. SUMMARY AND CONCLUSIONS

1. Eight simple recessive chlorophyll-deficient factors governing the formation of albino seedlings in red clover are reported. These have been designated *w*1, *w*2, *w*3, *w*4, *w*5, *w*6, *w*7 and *w*8.
2. Identity tests have shown that at least six of these factors, namely, *w*2, *w*3, *w*4, *w*5, *w*7 and *w*8, are genetically different. Tests with *w*1 and *w*6 are incomplete, but on the basis of the phenotypical appearance they are probably different from each other and from the other six *w* factors.
3. Most of these white seedling factors have also been shown to be genetically distinct from seven yellow lethal seedling factors reported in another paper in the current number of this journal.
4. Six of these factors, namely, *w*1, *w*2, *w*3, *w*4, *w*5 and *w*6 give rise to pure pale yellow seedlings which are rapidly bleached to pure white. These are lethal in the cotyledon leaf stage.
5. Genotypes *w*7 *w*7 and *w*8 *w*8 contain a small amount of chlorophyll on white ground. The *w*7 *w*7 seedlings are all lethal before the fourth leaf stage. Most of the *w*8 *w*8 seedlings perished in the early seedling stage but a few survived to maturity to become full green.
6. It appears probable from the aberrant ratios obtained that *w*1 is loosely linked with sterility allelomorphs *S*<sup>1</sup>, *S*<sup>2</sup>, etc.
7. *w*4, when present in the recessive condition, impedes the normal development of the seeds and acts partly as a pre-germination lethal as well as an early zygotic lethal. The results obtained suggest that *w*4 is weakly linked with *m*—a factor for no leaf-marking known to be located in linkage group I.
8. In addition to those determined by *w*1 to *w*8 factors, ten other white seedling types apparently due to simple recessive genes have been

found. The identity of these factors with each other and with w1 to w8 genes has not been tested.

9. A number of white seedlings giving widely aberrant ratios have also been observed.

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458 *Genetics of Chlorophyll Deficiencies in Red Clover*

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