Inheritance and Epistasis Studies of Chlorophyll Deficiency in Lettuce

E.J. Ryder,¹ Z.H. Kim,² and W. Waycott³

U.S. Department of Agriculture, Agricultural Research Service, 1636 East Alisal Street, Salinas, CA 93905

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ABSTRACT. Three mutant traits for chlorophyll deficiency in lettuce (*Lactuca sativa* L.), *bleached bud*, *calico-2*, and *pale green*, are inherited as single recessive alleles. *Bleached bud* is epistatic to another recessive allele, *dappled*. *Calico-2* is epistatic to *dappled*. *Pale green* is hypostatic to *chlorophyll deficient-3*. The Vanguard cd mutant is the same as *chlorophyll deficient-3*. The light green mutant 8744-1 is the same as *light green*. Independent inheritance is shown for *bleached bud* and *dappled*, *calico-2* and *dappled*, and *pale green* and *chlorophyll deficient-3*, respectively.

Genetic mapping in lettuce proceeds on two fronts. One is the generation of genetic markers of various types from a single cross by means of several molecular technologies (Kesseli et al., 1994). This enables the establishment of linkage between DNA-based markers and previously identified character genes (Waycott et al., 1999). The other is identification of new character genes, linkages, and epistatic effects by study of numerous crosses each involving relatively few traits. This report is of the latter type, and describes the inheritance of three new genes for chlorophyll deficiency, the epistatic relationship among these and other previously reported chlorophyll deficiency genes, and clarification of the identity of similar mutants.

The most recent publication on chlorophyll deficiency in lettuce described six mutants and their epistatic relationship to each other and to previously described mutants (Ryder, 1996). Of these, *chlorophyll deficient-3* (*cd-3*) is recessive and epistatic to *light green* (*lg*); *chlorophyll deficient-4* (*cd-4*) is recessive, hypostatic to *virescent* (*vi*), and linked to an anthocyanin gene; and *chlorophyll deficient-5* (*cd-5*), *chlorophyll deficient-6* (*cd-6*), and *chlorophyll deficient-7* (*cd-7*) are unlinked recessive genes. It was shown that *cd-5* and *cd-6* are both epistatic to *cd-7*. The sixth gene, *dappled* (*dap*), is recessive, lethal in combination with *sickly* (*si*), epistatic to *lg*, and hypostatic to *vi*.

Materials and Methods

The trait *bleached bud* occurs in a genetic line 92-217. This line was one of several mutant types isolated from early flowering lettuce treated with ethyl methane sulfonate (EMS) for the study of gibberellin regulated dwarfs (Waycott et al., 1995). In two crosses, 92-217 x 'Salinas' and 92-217 x 'Prizehead', the male parents were a standard crisphead cultivar and a standard red leaf cultivar, respectively. In four crosses, 92-163 x 92-217, 92-217 x 93-716-1 and reciprocal, and 93-716-28 x 92-217, the other parent was *dappled*.

Bleached bud may be described as follows: The cotyledons are green; the first true leaf and subsequent rosette leaves are blotchy green and yellow; cauline leaves are normal green; involucre are white when immature and turn light green with white tipped bracts as they mature.

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¹Present address: Petoseed Co., 650 Leanna Drive, Arroyo Grande, CA 93420.

²To whom reprint requests should be addressed. E-mail: ryder@pwa.ars.usda.gov

³Present address: Department of Horticulture, Gyeongsang National University, Jinju 660-701, South Korea.

Vanguard *cd* has yellow leaves, which are similar in appearance to those of 80-1277 (*cd-3*). The inheritance of Vanguard *cd* was studied in the cross Vanguard *cd* x 'Salinas 88'. Vanguard *cd* was found in a greenhouse population of 'Vanguard', a crisphead lettuce. The cross 80-1277 x Vanguard *cd* was made to test for allelism.

Calico was selected from a field population of mutants derived from treatment with EMS. It was used in the following crosses for genetic analysis: Calico x 'Prizehead', Calico x Pseudo-tipburn, Calico x GL 77, Calico x 'Oakleaf', 92-163 x Calico, Calico x 80-1277 and reciprocal, and Calico x Vanguard *cd* and reciprocal. Pseudo-tipburn is a mutant type not reported here. GL 77 is a leaf type lettuce line from Korea. 'Oakleaf' is a leaf type cultivar.

Calico has green cotyledons with white margins. The true leaves show a mosaic of dark and light green, yellow-green, and white. Later appearing leaves, including the cauline leaves, are green with no mosaic effect. The involucre are white when immature, turning light green with maturity; this phenotype is very similar to *bleached bud*.

Pale green also was derived from a population treated with the mutagen and was originally called EMS yellow. It was studied in the cross 80-1277 x EMS yellow. Cotyledons are green. True leaves on young plants are very light green with a yellowish cast, becoming greenish-yellow on new leaves forming on older plants. Involucre on the seed head are greenish-yellow. Pedicels are short, giving the seed head a compressed appearance.

'Australian' and 8744-1 are both light green in color, with no apparent color change as the plant matures. 'Australian' is a leaf lettuce with lobed leaves. Leaf color is controlled by *light green* (*lg*) (Lindqvist, 1960; Ryder unpublished; Thompson, 1938). 8744-1 is a mutant derived from seeds treated with thermal neutrons. The cross 8744-1 x 'Australian' was made to study gene identity.

All new genes reported herein were studied in F₂ populations and F₃ families. All segregations were analyzed using χ^2 comparisons.

Results and Discussion

Two groups of populations were grown and analyzed for the inheritance of *bleached bud*. The first included two crosses of 92-217 with 'Salinas' and 'Prizehead', respectively. Segregation of the *bleached bud* trait only was tracked. The F₁ populations were green. In both F₂ populations, segregation of green:*bleached bud* approximated closely to a 3:1 ratio (Table 1). Among F₃ families from green F₂ plants, the ratio of green:segregating families approximated 1:2. Within segregating F₃ families, the ratio of

green:bleached bud plants approximated 3:1. Anthocyanin content contributed by 'Prizehead' was not affected by the chlorophyll deficient trait, nor did it mask the deficiency.

The second group consisted of four populations from crosses between the bleached bud parent (92-217) and plants with the trait *dappled* (92-163, 93-716-1, 93-716-28). F₁ plants were green. The F₂ populations segregated approximately 9 green:3 dappled:4 bleached bud (Table 2). One population fit less well than the other three, but heterogeneity was not significant ($\chi^2 = 5.55$, $P = 0.1-0.2$). Among F₃ families from green or *dappled* F₂ plants, the expected distribution of families was 1:2:2:4:2:1 for all green; green and dappled; green and bleached bud; green, dappled, and bleached bud; dappled and bleached bud; and all dappled. This expectation was met (Table 2). Finally, segregation within segregating F₃ families approximated 3:1 for single gene segregation. In those families where both genes were segregating, the expectation of 9:3:4 was barely met ($\chi^2 = 6.66$, $P = 0.02-0.05$), because

of an excess of *dappled* plants and a shortage of bleached bud plants.

The preponderance of evidence indicates that bleached bud is inherited as a single recessive and the gene is named *bleached bud* (*Blbl*). It is epistatic to *dappled*.

The Vanguard cd mutant was studied in the cross Vanguard cd x 'Salinas 88'. The F₁ was green. In F₂, the ratio of green: chlorophyll deficient approximated 3:1 (Table 3). F₃ family segregation from green plants approximated 1 green:2 segregating. Segregation within segregating families approximated 3:1. Results indicate a single gene with chlorophyll deficiency recessive. The similarity of the phenotype to that of *cd-3* suggested making the cross 80-1277 x Vanguard cd and its reciprocal. All F₁ plants were yellow in both crosses and there was no segregation in F₂. Therefore, it is highly likely that the Vanguard cd mutant is *cd-3* and the latter designation will be used in the remainder of the paper.

Table 1. Segregation ratios of the chlorophyll deficient mutant bleached bud in two lettuce populations.

	Observed no.		χ^2	<i>P</i>
	Green	bb		
F ₂ (Exp. 3:1)				
92-217 x 'Salinas'	51	20	0.39	0.5-0.7
92-217 x 'Prizehead'	62	21	0.004	0.95
F ₃ families	Green	Segr.		
Among families from green F ₂ (Exp. 1:2)				
92-217 x 'Salinas'	18	22	2.46	0.1-0.2
92-217 x 'Prizehead'	11	26	0.21	0.5-0.7
Within segregating families (Exp. 3:1)				
92-217 x 'Salinas'	260	81	0.28	0.5-0.7
92-217 x 'Prizehead'	279	95	0.03	0.8-0.9

Table 2. Segregation ratios of chlorophyll deficient mutants, bleached bud and dappled, in four lettuce populations.

	Observed no.			χ^2	<i>P</i>
	Green	Dappled	bb		
F ₂ (Exp. 9:3:4)					
92-163 x 92-217	166	52	68	0.38	0.8-0.9
92-217 x 93-716-1	135	50	80	4.18	0.1-0.2
93-716-1 x 92-217	163	52	68	0.18	0.9-0.95
93-716-28 x 92-217	164	59	66	0.98	0.5-0.7
Among F ₃ families, 92-163 x 92-217 (Exp. 1:2:2:4:2:1)		Observed	Expected	χ^2	<i>P</i>
From green F ₂ plants					
All green		6	7.92	0.47	
Green:dappled		18	15.83	0.30	
Green:bleached bud		10	15.83	2.15	
Green:dappled:bleached bud		33	31.67	0.06	
From dappled F ₂ plants					
Dappled:bleached bud		21	18.67	0.29	
All dappled		7	9.33	0.58	
Total				4.78	0.3-0.5
	Observed no.			χ^2	<i>P</i>
	Green	Dappled	bb		
Within segregating families (Exp. 3:1, 9:3:4)					
Green:bleached bud	245		68	1.79	0.1-0.2
Dappled:bleached bud		334	115	0.09	0.7-0.8
Green:dappled	569	210		1.59	0.2-0.3
Green:dappled:bleached bud	693	256	273	6.66	0.02-0.05

Three groups of populations were grown and analyzed for the inheritance of calico. The first group included populations from the crosses: Calico x 'Prizehead', Calico x Pseudo-tipburn, Calico x GL 77, and Calico x 'Oakleaf'. Only the calico trait was studied. F₁ plants were green. In three F₂ populations, segregation for green:calico gave acceptable fits to a 3:1 ratio (Table 4). No heterogeneity was shown ($\chi^2 = 1.72$, $P = 0.3-0.5$). In the fourth F₂ population, there was a severe shortage of calico plants. The only explanation that could be advanced was the failure of 25 seeds to germinate; if all or nearly all were calico, the fit to 3:1 would be improved. Segregation among F₃ families approximated 1:2 or 1:2:1, and within segregating families approximated 3:1, as expected.

The second group consisted of populations segregating for calico and a second trait. One, 92-163 x Calico, segregated for *dappled* as well as calico. The F₁ was green. The F₂ segregated approximately 9 green:3 *dappled*:4 calico, suggesting that calico is inherited as a single recessive and is epistatic to *dappled* (Table 5). Among F₃ families, family distribution approximated 1:2:2:4:2:1:4 for all green; green and *dappled*; green and calico; green, *dappled*, and calico; *dappled* and calico; all *dappled*; and all calico. Segregation within segregating F₃ families approximated 9 green:3 *dappled*:4 calico; 3 green:1 *dappled*; 3 green:1 calico; and 3 *dappled*:1 calico, respectively, as expected.

The third group consisted of four populations, Calico x 80-1277 and its reciprocal and Calico x Vanguard cd and its reciprocal, which segregated for calico and *cd-3*. F₁ plants were green. Two F₂ populations segregated approximately 9 green:3 *cd-3*:3 calico:1 light green calico (Table 6). The F₂ of 80-1277 x Calico segregated with an excess of both the *cd-3* and light green calico phenotypes. The other reciprocal population, Vanguard cd x Calico, showed a severe shortage of the light green calico class.

F₃ family data were taken from the Calico x Vanguard cd and reciprocal populations. In F₃ families the light green calico and calico classes were difficult to separate and were counted as one class, calico. Among F₃ families from green plants, both sets approximated 1:2:2:4 for all green; green and *cd-3*; green and calico; green, *cd-3*, and calico. Within segregating families, for both populations, segregation was as expected. As calico and light green calico were counted together as one class, segregation in those families, where both mutants were segregating, approximated 9 green:3 *cd-3*:4 calico. This is not epistasis, since the traits can be distinguished.

Whitaker (1968) described a chlorophyll deficient mutant, which he did not name. It was named *calico* later (Robinson et al., 1983) and symbolized *Clcl*. This mutant was subsequently lost and could not be compared to the one we describe. However, the description differed from the one described in this study; the cotyledons were light green and true leaves had patches of green and white tissue. Therefore, it was likely not the same mutant. Our calico is inherited as a single recessive and is named *calico-2* (*Cl-2cl-2*).

Inheritance of pale green was studied, together with *cd-3*, in the cross 80-1277 x EMS yellow. The F₁ was green. In one F₂ population, segregation of green:pale green approximated 3:1, and segregation of green:pale green:*cd-3* approximated 9:3:4 (Table 7). In a second F₂ population, the plants were crowded in the flat and many died, which probably accounts for a poor fit to the expected 9:3:4. Among F₃ families from green plants of both F₂ populations, the ratio approximated 1:2:2:4 for all green; green and pale green; green and *cd-3*; and green, pale green, and *cd-3*. Among families from pale green plants, the ratio approximated 1:2:1 for all pale green; pale green and *cd-3*; and all *cd-3*. Within segregating families, ratios approximated 3:1 for green and pale green; green and *cd-3*; and pale green and *cd-3* and approximated

Table 3. Segregation ratios of chlorophyll deficiency in the cross Vanguard cd x 'Salinas 88'.

	Observed no.			χ^2	<i>P</i>
	Green	Segregating	cd		
F ₂ (Exp. 3:1)	78		20	1.11	0.2-0.3
Among F ₃ families from green plants (Exp. 1:2)	12	39		2.21	0.1-0.2
Within segregating F ₃ families (Exp. 3:1)	603		177	2.21	0.1-0.2

Table 4. Segregation ratios of chlorophyll deficient mutant calico, in four lettuce populations.

	Observed no.			χ^2	<i>P</i>
	Green		Calico		
F ₂ (Expect 3:1)					
Calico x 'Prizehead'	110		25	3.03	0.05-0.1
Calico x Pseudo-tipburn	81		26	0.03	0.9-0.95
Calico x GL 77	186		37	9.41	<.01
Calico x 'Oakleaf'	168		54	0.05	0.8-0.9
F ₃ families	Green	Segr.	Calico		
Among families from green F ₂ (Exp. 1:2)					
Calico x Pseudo-tipburn ^a	54	127		0.99	0.3-0.5
Among families from F ₂ plants (Exp. 1:2:1)					
Calico x 'Prizehead'	13	23	11	0.19	0.9-0.95
Within segregating families (Exp. 3:1)					
Calico x Pseudo-tipburn	2049	692		0.09	0.7-0.8
Calico x 'Prizehead'	194	81		2.91	0.05-0.1

^aNumber of F₃ families include 79 from 81 green F₂ plants shown plus 112 F₃ families from another F₂ not evaluated.

Table 5. Segregation ratios of calico and dappled in the cross 92-163 x Calico.

	Observed no.			χ^2	<i>P</i>
	Green	Dappled	Calico		
F ₂ (Exp. 9:3:4)	175	42	73	3.68	0.1–0.2
Among F ₃ families (Exp. 1:2:2:4:2:1:4)	Observed	Expected		χ^2	<i>P</i>
From green F ₂ plants					
All green	2	5.75		2.45	
Green:dappled	14	11.5		0.54	
Green:calico	15	11.5		1.07	
Green:dappled:calico	27	23.0		0.70	
From dappled F ₂ plants					
Dappled:calico	11	11.5		0.02	
All dappled	7	5.75		0.27	
From calico F ₂ plants					
All calico	16	23.0		2.13	
Total				7.18	0.3–0.5
	Observed no.			χ^2	<i>P</i>
	Green	Dappled	Calico		
Within segregating families (Exp. 9:3:4, 3:1)					
Green:dappled:calico	605	210	255	1.04	0.5–0.7
Green:dappled	265	108		3.11	0.05–0.1
Green:calico	462		159	0.12	0.7–0.8
Dappled:calico		282	102	0.51	0.3–0.5

Table 6. Segregation ratios for calico and cd-3 in four lettuce populations.

F ₂ (Exp. 9:3:3:1)	Green	cd-3	Calico	Both	χ^2	<i>P</i>
Calico x 80-1277	127	55	43	16	2.84	0.3–0.5
80-1277 x Calico	159	72	43	25	10.20	0.01–0.02
Calico x Vanguard cd	156	48	47	12	1.78	0.5–0.7
Vanguard cd x Calico	160	40	48	7	8.20	0.02–0.05
Calico x Vanguard cd and reciprocal		Cal x Van cd		Reciprocal		
Among F ₃ families from green F ₂ plants (Exp. 1:2:2:4)	Obs.	Exp.	χ^2	Obs.	Exp.	χ^2
All green	4	3.56	0.05	3	3.89	0.02
Green:cd-3	6	7.11	0.17	6	7.78	0.03
Green:calico	8	7.11	0.11	4	7.78	0.92
Green:cd-3:calico ^z	14	14.22	0	16	15.56	0.75
Total χ^2			0.33			1.72
<i>P</i>			0.95–0.98			0.5–0.7
Within segregating families (Exp. 9:3:4, 3:1)	Cal x Van cd			Reciprocal		
	Obs. no.	χ^2	p	Obs. no.	χ^2	p
Green	237	0.77		257	1.10	
cd-3	60	2.86		76	0.23	
Calico ^z	101	0.02		95	1.35	
Total		2.92	0.3–0.5		2.68	0.2–0.3
Green	137	0.09		134	0.14	
cd-3	41	0.27		39	0.42	
Total		0.36	0.5–0.7		0.56	0.3–0.5
Green	172	0		89	0.11	
Calico	57	0		34	0.34	
Total		0	>0.99		0.45	0.5–0.7

^zIncludes calico and light green calico classes due to difficulty in separating the two classes.

Table 7. Segregation ratios for pale green and chlorophyll deficient-3 in the cross 80-1277 (cd-3) x EMS yellow (pale green).

F ₂ population	Observed no.			χ^2	P
	Green	pale green	cd-3		
Exp. 3:1	98	23		2.32	0.1–0.2
Exp. 9:3:4	98	23	31	4.18	0.1–0.2
Exp. 9:3:4	311	72	96	14.66	<0.01
Among F ₃ families					
From green plants (Exp. 1:2:2:4)	Observed	Expected		χ^2	P
All green	2	3.89		0.92	
Green:pale green	9	7.78		0.19	
Green:cd-3	6	7.78		0.41	
Green:pale green:cd-3	18	15.56		0.38	
Total				1.90	0.5–0.7
From pale green plants (Exp. 1:2)					
All pale green	24	23.67		0.33	
Segregating	47	47.33		0.33	
Total				0.66	0.3–0.5
Within segregating families (Exp.9:3:4, 3:1)					
	Green	pale gr.	cd-3	χ^2	P
Green:pale green:cd-3	216	86	102	1.96	0.3–0.5
Green:pale green	166	58		0.09	0.7–0.8
Green:cd-3	100		45	2.81	0.05–0.1
pale green:cd-3		680	245	1.08	0.2–0.3

9:3:4 for green:pale green:cd-3.

The conclusion from these results is that pale green is inherited as a single recessive. The gene is named *pale green* and is symbolized *Pgpg*. It is hypostatic to *cd-3*.

F₁ and F₂ progeny of the cross 8744-1 x 'Australian' were all light green in color with no apparent variation. It was concluded that the mutant allele in 8744-1 was *light green (lglg)*, identical to that in 'Australian'.

The joint segregations of *dappled* and *bleached bud, calico-2* and *dappled*, and *cd-3* and *pale green* showed no deviations from independent inheritance. The number of individuals was low for a linkage study, so at this point the tentative conclusion is that there is no linkage between these pairs.

Information on the inheritance of these traits impacts primarily in their contribution to the lettuce genetic map and in potential physiology studies. As chlorophyll deficient mutants, they are less likely to have usefulness in a breeding program except as novelties.

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