Aneuploid analyses of three chlorophyll abnormalities in Emmer wheat

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Of the various chlorophyll abnormalities that occur in polyploid wheats, genetic bases of only two types, chlorina and virescence, are known. Here, for the first time, the chromosomal bases of three other chlorophyll abnormalities, striato-virescence, delayed virescence, and albino, which occur in Emmer wheat ($2n = 4x = 28$, genome constitution AABB) are reported. A set of disomic substitution lines of Langdon durum was used for chromosome identification. All three abnormalities are controlled by two duplicated recessive genes (carrier chromosome in parentheses); striato-virescence by *sv1* (3A) and *sv2* (2A), delayed virescence by *dv1* (2B) and *dv2* (supposedly 2A), and albino by *abn1* (2A) and *abn2* (2B). Genes on the same chromosome are located in different loci and are recombined with each other. The Chinese Spring cultivar of common wheat ($2n = 6x = 42$, genome constitution AABBDD) carries wild-type homoeoalleles for these abnormalities; *Sv3* (2D), *Dv3* (2D), and *Abn3* (2D).

Key words: albino, aneuploid analysis, delayed virescence, polyploid wheats, striato-virescence

INTRODUCTION

Two true-bred lines that have the AABB genome constitution and *ne1ne1 Ne2Ne2* genotype were obtained by transferring the *Ne2* gene (one of two complementary genes for hybrid necrosis; Tsunewaki, 1960) from the common wheat 'Jones Fife' (abbreviated JF) to the emmer wheat 'Vernal'. One line stably expressed a chlorophyll abnormality known as striato-virescence. During the genetic analysis of this abnormality, two other chlorophyll abnormalities, delayed virescence and albino, appeared. Lines fixed for the genes of those abnormalities were bred and subjected to aneuploid analysis using a set of disomic substitution lines of the durum wheat 'Langdon' (abbrev. Ldn DS lines), in which the Ldn's A- or B-genome chromosome is substituted by its D-genome homoeologue of the common wheat cultivar 'Chinese Spring' (abbrev. CS) (Joppa and Williams, 1988).

Striato-virescence was reported by Frankel (1950), but the chromosome basis of its inheritance is unknown (McIntosh et al., 1998). Delayed virescence is a new chlorophyll abnormality. Albino is a chlorophyll mutation frequently seen by wheat workers, but so far no locus has been identified for it due to the lethality of albinotic

plants. Aneuploid analyses of these three chlorophyll abnormalities therefore have been made.

The findings reported here provide new information on the genetic bases of chlorophyll abnormalities in wheat. Moreover, they show the limitation of aneuploid analysis that uses DS lines for traits determined by duplicated recessive genes.

MATERIALS AND METHODS

Designation of wheat genotypes and chromosomes

The following names are used here to indicate different wheat genotypes: 'Emmer wheat' for tetraploid genotypes having the AABB genome constitution ($2n = 4x = 28$) and 'common wheat' for hexaploid genotypes of the AABBDD genome constitution ($2n = 6x = 42$). Cultivars of *T. dicoccum* and *T. durum*, both belonging to Emmer wheat, respectively are called emmer and durum wheat.

Designations of the wheat chromosomes used in this study follow the recommendations of the 7th International Wheat Genetics Symposium (Miller and Koebner, 1988). The former 4A is renamed 4B, and the former 4B renamed 4A.

Breeding mutant stocks

Striato-virescence. The abbreviation 'sv' is used for this phenotype, and the basic symbol, *sv*, adopted for its genes. This abnormality is characterized by normal green seedling leaves in autumn, albinotic stripes on

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rosette leaves in winter (Fig. 1A, right), and recovery of normal leaf color in spring. When *sv* homozygotes are grown in a greenhouse adjusted at 15°C or higher temperature, they do not express this abnormality.

Two tetraploid lines ($2n = 28$) homozygous for *Ne2* genes were bred by transferring this gene from common wheat JF to the Emmer (Vernal) background. One line, named *sv Ne2-4x*, stably expressed the *sv* phenotype.

Fig. 1. (A): Rosette stage of a normal (left), delayed virescent (center), and striato-virescent plant (right). (B): Normal (four seedlings at left) and albinotic plants (four seedlings at right) segregated in an F_4 progeny of the cross Ldn x (Vernal x *sv Ne2-4x*) F_1 .

Because Ldn carries *Ne1* which causes hybrid necrosis in the presence of *Ne2* (Tsunewaki, 1960), breeding an *Ne2*-free *sv* homozygote was necessary to determine the chromosomal locations of *sv* genes by means of aneuploid analysis with use of Ldn DS lines. This was done by selecting *sv* homozygotes in the offspring of the cross, Ldn x (Vernal x *sv Ne2-4x*) F₁, in which Vernal is free of both *Ne1* and *Ne2*. The *sv*-homozygous line obtained that is free of *Ne2* was named *sv ne2-4x*.

Delayed virescence. The abbreviation for this phenotype is 'dv'. The basic symbol *dv* is used for its genes. This phenotype is characterized by normal green-colored seedlings in autumn, which turn almost completely albino in winter (Fig. 1A, center). Most *dv* seedlings die at that stage, but a few survive through winter. Those that do gradually recover chlorophyll and growth vigor in the spring and finally set seeds. Their maturity, however, is about one month later than that of normal plants. When *dv* homozygotes are grown in a greenhouse adjusted at 15°C or higher temperature, they do not show delayed virescence, although their growth vigor is subnormal. A few fixed *dv* lines free of *Ne2*, obtained in the offspring of the cross Ldn x (Vernal x *sv Ne2-4x*) F₁, were used in the present analysis.

Albino. The abbreviation for this phenotype is 'abn'. The basic symbol *abn* is used for its genes. Albino seedlings lack chlorophyll from the time of germination (Fig. 1B), are pure white (or slightly purple when anthocyanin is present), and die at about the third leaf stage when the starch in the attached seeds is exhausted. Because albinos are completely lethal, no fixed albino lines were obtained. True-bred lines for each of the duplicated (or complementary) recessive *abn* genes therefore were selected from the offspring of the cross Ldn x (Vernal x *sv Ne2-4x*) F₁, and used for *abn* gene identification. Details of the procedure for their breeding are given in the corresponding section in "RESULTS".

Aneuploid analysis

A complete set of the 14 Ldn DS lines produced by Joppa and Williams (1988) was used. Each DS line is indicated by its D-genome chromosome, the replaced A- or B-genome homoeologue being given in parentheses. For example, Ldn 1D(1A) is a DS line in which a pair of Ldn 1A chromosomes is replaced by a pair of CS 1D chromosomes. Of the 14 DS lines, 4D(4A), 5D(5A), and 6D(6B) respectively retained a single 4A chromosome, single 5AL arm, and single 6BS arm.

In a preliminary study, the 14 DS lines and a normal Ldn line, the control, were crossed as female to (Vernal x *sv Ne2-4x*) F₁'s. For convenience, seeds obtained from these crosses are hereafter called F₁ seeds. Open-pollinated F₂ seeds were harvested from 30 F₁ plants of each

DS line and 40 F₁'s of the control, but the 3D(3B) line gave only 11 F₁ plants. The F₂ seeds were sown in flats in a greenhouse, and the germination rate and frequency of albinos examined. All the green seedlings were transplanted to the field in late autumn. Segregation of *dv* and *sv* plants was examined a few times during the winter.

In the advanced studies, the same set of DS lines and a normal Ldn line were crossed to *sv ne2-4x*, to the *dv* homozygote, and to the homozygote for each of the two complementary *abn* genes. F₁ plants were grown in pots in a greenhouse, and F₂ seeds obtained by self-pollination were sown in flats also in a greenhouse where segregation of albinos was observed. In early winter, the flats were taken outside. Segregation of *dv* and *sv* plants in the individual F₂ progenies was recorded a few times during the winter.

RESULTS

Preliminary study

As expected, the F₁ populations of all the DS and control lines, except the Ldn 5D(5B) line, segregated normal and necrotic plants in about a 1 : 1 ratio. Line Ldn 5D(5B) lacks the 5B chromosomes of Ldn which carry *Ne1*. All the offspring therefore should be non-necrotic. In fact, 137 plants were normal, two others necrotic. The origins of these off-type plants are unknown. All the normal hybrids are assumed to be homozygous for *ne2*. All the parental lines in the advanced studies were descendants of those normal F₁'s.

All three chlorophyll abnormalities segregated (Table 1) in F₂ offspring of the normal F₁'s. Several DS lines clearly showed segregation ratios for each chlorophyll abnormality that differed from those of the disomic population. It was, however, difficult to definitively determine which chromosomes carry the responsible genes. The reasons were (1) frequencies of all the abnormalities were low in the disomic (control) population, (2) co-segregation of all three abnormalities occurred, and interaction was suspected, (3) the *Ne2* gene appeared to be linked to some chlorophyll abnormality genes. For a clear identification of the carrier chromosomes of the responsible genes, advanced studies therefore were made on fixed lines for each abnormality, which lines were free of the other abnormality genes.

Advanced study of striato-virescence

A fixed line for this abnormality, free of *Ne2* and other chlorophyll abnormality genes, was crossed to Ldn DS lines and to a normal line (the control). F₂ populations obtained by the self-pollination of F₁ hybrids were examined for segregation of *sv* plants (Table 2). The frequency in most lines was close to 6.25% (= 1/16), indicative that two duplicated recessive genes determine the expression of this phenotype. The postulated genes were assigned

Table 1. Segregation of three chlorophyll abnormalities in the F₂ generation of the crosses Ldn DS x (Vernal x *sv Ne2-4x*)F₁.

F ₂ line	No. progenies	No. seeds sown	No. seeds germinated	No. albinos	% albinos	No. surviving plant			% sv	% dv
						Total	sv	dv		
1D(1A)	30	1,200	1,171	30	2.56	1,127	18	7	1.60	0.62
2D(2A)	30	1,200	1,156	0	0.00**	1,152	18	0	1.56	0.00
3D(3A)	30	1,200	1,144	26	2.27	1,096	68	4	6.20**	0.36
4D(4A)	30	1,200	1,148	32	2.79	1,095	23	18	2.10	1.64**
5D(5A)	30	1,200	1,119	6	0.54**	1,092	29	0	2.66	0.00
6D(6A)	30	1,200	1,141	14	1.25*	1,106	38	13	3.44*	1.18*
7D(7A)	30	1,200	1,165	15	1.29*	1,111	21	12	1.89	1.08*
1D(1B)	30	1,200	1,136	27	2.38	1,100	14	6	1.27	0.55
2D(2B)	30	1,200	1,143	39	3.41	1,085	2	7	0.18**	0.65
3D(3B)	11	1,032	940	13	1.38	897	9	7	1.00*	0.78
4D(4B)	30	1,200	1,150	30	2.61	1,093	38	6	3.48*	0.55
5D(5B)	30	1,200	1,135	9	0.79**	1,106	27	15	2.44	1.36**
6D(6B)	30	1,200	1,145	18	1.57	1,098	23	26	2.09	2.37**
7D(7B)	30	1,200	1,156	10	0.87**	1,128	12	10	1.06*	0.89
Disomic	40	1,600	1,568	37	2.36	1,531	32	5	2.09	0.33
Total ¹⁾										
For sv	—	—	—	—	—	13,484	284	—	2.11	—
For dv	—	—	—	—	—	14,580	—	129	—	0.88
For abn	—	15,118	267	—	1.77	—	—	—	—	—

1) Total excluding respective critical lines; 2D(2A), 3D(3A), and 2D(2B) for sv, and 2D(2A) and 2D(2B) for both dv and abn
 * and **: Significant respectively at the 5% and 1% levels of probability

Table 2. Frequency of striato-virescence (sv) in the F₂ generation of the crosses Ldn DS's x *sv ne2-4x*.

F ₂ line	No. progenies	No. of seeds		No. of plants		% sv	χ^2 -value (gr:sv=15:1)	χ^2 -value (vs.disomic)
		Sown	Germinated	Green	Striato-v.			
1D(1A)	4	200	200	186	14	7.00	0.19	3.64
2D(2A)	4	200	200	190	10	5.00	0.53	0.77
3D(3A)	4	200	197	177	20	10.15	5.12*	10.82**
4D(4A)	4	200	200	187	13	6.50	0.02	2.76
5D(5A)	4	200	199	190	9	4.52	1.02	0.37
6D(6A)	4	200	199	185	14	7.04	0.21	3.69
7D(7A)	4	200	199	188	11	5.53	0.18	1.35
1D(1B)	4	200	200	192	8	4.00	1.73	0.09
2D(2B)	6	200	199	197	2	1.01	9.35**	3.19
3D(3B)	4	200	186	174	12	6.45	0.01	2.58
4D(4B)	4	200	198	188	10	5.05	0.49	0.82
5D(5B)	4	200	200	190	10	5.00	0.53	0.77
6D(6B)	7	200	197	190	7	3.55	2.44	0.00
7D(7B)	4	200	200	192	8	4.00	1.73	0.09
Disomic	4	400	399	385	14	3.51	5.12*	—
Pooled ¹⁾	55	2,800	2,577	2,447	130	5.04	6.39*	—

1) All progenies, except those of the 2D(2A), 3D(3A), and 2D(2B) lines, are pooled.
 * and **: Significant respectively at the 5% and 1% levels of probability

the symbols *sv1* and *sv2*.

Disomic and pooled data indicated that the frequency of *sv* plants is slightly, but significantly lower than the expected 6.25%. This deviation is not attributable to female sterility because female fertility of the disomic F_1 's was normal (average 94%). One possible explanation is that there is a slight disadvantage (estimated to be about 5%) in fertilization of pollen of the *sv1sv2* genotype as compared to pollen having the *Sv1* and/or *Sv2* allele.

Based on the 15 green to 1 *sv* ratio, a χ^2 -test was made (second column at right, Table 2). F_2 progenies of two DS lines, Ldn 3D(3A) and Ldn 2D(2B), had ratios that deviated significantly from the expected value. In the Ldn 3D(3A) progenies, the frequency of *sv* plants was about two times the expected 6.25%. The conclusion is that one of the duplicated genes, *sv1*, is located on chromosome 3A of *sv ne2-4x*. According to Joppa and Williams (1988, Table 5), about 41% of the F_2 offspring of 3A/3D double monosomics have a pair of 3A chromosomes. Therefore, about 10% (i. e., $41\% \times 1/4$) of the F_2 offspring of the cross, Ldn 3D(3A) $\times$ *sv ne2-4x*, are expected to have the *sv* phenotype, assuming that a double dose of *sv1*, in addition to the homozygous condition of *sv2*, is necessary for the *sv* phenotype. The actual frequency of 10.2% for offspring of 3D(3A) lines fits that frequency.

The extremely low frequency of *sv* plants in the F_2 progenies of Ldn 2D(2B) suggests that chromosome 2B or 2D carries an *sv* suppressor. The parental F_1 's, Ldn 2D(2B) $\times$ *sv ne2-4x*, differ from the control F_1 's in the following two respects. One is the presence of the 2B chromosome in a single dose, as compared to the double dose in the control. The other is the presence of the 2D chromosome that is lacking in the control. Because the 2B chromosome in the Ldn 2D(2B) $\times$ *sv ne2-4x* F_1 's was derived from the *sv ne2-4x* parent, it can not be the carrier of the *sv* suppressor. Therefore, chromosome 2D must carry the suppressor. According to the findings of Joppa and Williams (cited above), the frequency of 2D-chromosome carriers in the F_2 offspring of 2B/2D double monosomics is about 34%, close to $1/3$. The frequency of *sv* plants in the F_2 's of the cross Ldn 2D(2B) $\times$ *sv ne2-4x* therefore is expected to be about $1/4 \times 1/4 \times 1/3$ (ca. 2.1%). The observed frequency of 1.0% fits the expected ratio ($\chi^2 = 0.670$, $df = 1$).

Based on the above deduction, F_2 progenies of the cross Ldn 2D(2A) $\times$ *sv ne2-4x* should segregate *sv* plants at a much lower frequency than do disomic F_2 's and should show an *sv* plant frequency similar to that of the cross Ldn 2D(2B) $\times$ *sv ne2-4x* because they have the 2D chromosome in common. This, however, was not the case. They segregated *sv* plants at a frequency similar to that of the disomic F_2 's, indicative that chromosome 2A of *sv ne2-4x* carries the *sv2* gene; the other *striato-virescence* gene. According to Joppa and Williams (cited above), the frequency of F_2 plants that have a double dose of the 2A

chromosome (accordingly, a double dose of the *sv2* gene) but which lack the 2D chromosome (therefore being free of the *sv* suppressor) is about 35%. The frequency of *sv* plants in F_2 progenies of the cross Ldn 2D(2A) $\times$ *sv ne2-4x* therefore is expected to be about $0.35 \times 1/4$, about 8.7%. This fits the observed frequency of 5.0% ($\chi^2 = 3.522$, $df = 1$).

The *sv* suppressor is assumed to be a wild-type homoeoallele of the *sv2* gene as they are located on the 2D and 2A chromosomes respectively in the same homoeologous group. This suppressor therefore is designated *Sv3*. The fact that the frequency of *sv* plants in the preliminary study (2.1%, ref. Table 1) was about half that in the present study (5.0%) is explained by the genotypic difference of the male parents crossed to Ldn DS lines; *Sv1sv1 sv2sv2* in the preliminary study (ref. Table 1) and *sv1sv1 sv2sv2* in this one.

Advanced study of delayed virescence

Fixed lines with delayed virescence, free of *Ne2* and other chlorophyll abnormality genes, were crossed to Ldn DS lines, and the F_1 hybrids self-pollinated. F_2 offspring were tested for the segregation of *dv* plants (Table 3).

The frequency of *dv* plants was close to 25% in about 1/3 of the DS lines, but all the lines had frequencies lower than 25%. Control F_1 's were fully fertile on the female side (97% selfed seed fertility), but the F_2 progenies had frequencies of *dv* plants lower than 25%. This indicates that there is a fertilization disadvantage in having *dv*-carrying pollen as opposed to having *Dv*-carrying pollen (the estimated disadvantage is ca. 14%). According to the F_2 segregation ratio, the Ldn and *dv* homozygotes appear to differ by a single gene pair.

Segregation ratios of *dv* plants in F_2 progenies of the DS lines were tested against the control F_2 (disomic) ratio because the frequency of *dv* plants in the controls statistically was lower than 25%. F_2 progenies of two DS lines, Ldn 2D(2A) and Ldn 7D(7A), had ratios that deviated significantly from the disomic ratio; lower in the former, higher in the latter. Ldn 2D(2B) progenies did not differ from the disomic ones. These findings suggest that the 2D chromosome carries a suppressor for *dv* expression and that the 2B chromosome has a *dv* gene. If not, the F_2 progenies of Ldn 2D(2B) would segregate *dv* plants at a low frequency similar to that of Ldn 2D(2A). The *dv* frequency in F_2 progenies of Ldn 7D(7A) was 22.1%, only a little lower than 25%, indicative that chromosome 7A has no relation to the *dv* phenotype.

Two conclusions are drawn: (1) delayed virescence is controlled by a single gene, *dv1*, located on chromosome 2B of the *dv* homozygote (consequently, the 2B of Ldn has its wild-type allele, *Dv1*); (2) chromosome 2D of CS carries a *dv* locus on which a wild-type homoeoallele of *dv1is* located. This wild-type homoeoallele is designated *Dv3* because of its presence in the third wheat genome, D.

Table 3. Segregation of delayed virescence (dv) in the F₂ generation of the crosses Ldn DS's x *dv* homozygote.

F ₂ line	No. progenies	No. of seeds		No. of plants		%	χ^2 -value (vs. disomic)	χ^2 -value (gr:dv=3:1)
		Sown	Germinated	Green	dv			
1D(1A)	4	200	200	163	37	18.5	0.87	4.51*
2D(2A)	4	200	200	183	17	8.5	5.44*	29.04**
3D(3A)	4	200	200	166	34	17.0	0.23	6.83**
4D(4A)	4	200	200	176	24	12.0	1.23	18.03**
5D(5A)	4	200	200	166	34	17.0	0.23	6.83**
6D(6A)	4	200	200	170	30	15.0	0.02	10.67**
7D(7A)	4	240	240	187	53	22.1	4.24*	1.09
1D(1B)	4	200	200	163	37	18.5	0.87	4.51*
2D(2B)	4	200	200	158	42	21.0	2.74	1.71
3D(3B)	4	200	200	156	44	22.0	3.75	0.96
4D(4B)	4	200	200	156	44	22.0	3.75	0.96
5D(5B)	4	200	200	159	41	20.5	2.29	2.16
6D(6B)	6	113	113	89	24	21.2	2.06	0.85
7D(7B)	4	200	200	167	33	16.5	0.11	7.71**
Disomic	7	350	350	296	54	15.4	—	—
Pooled ¹⁾	—	2,703	2,703	2,214	489	18.1	—	68.81**

1) All pooled except the 2D(2A) and 2D(2B) lines

* and **: Significant respectively at the 5% and 1% levels of probability

Based on these assumptions, the respective frequencies of *dv* plants in the F₂ progenies of the two critical DS lines Ldn 2D(2A) and Ldn 2D(2B) should be 6.2% and 25.7%, close to their observed frequencies of 8.5% and 21.0%.

As stated hereafter (ref. Table 6), the F₂ population of the cross Vernal x Ldn produced some *dv* plants, indicative that each parent has one or the other duplicated recessive *dv* gene. Consistent with the previous conclusions, the genotype of Ldn must be *Dv1Dv1 dv2dv2* and that of Vernal *dv1dv1 Dv2Dv2*. Although the carrier chromosome of the *dv2* locus was not determined in this study, that locus is assumed to be on the 2A chromosome based on the homoeology of the 2A chromosome to both the 2B and 2D chromosomes, carriers of the *dv1* and *dv3* loci, respectively. The *dv* homozygote should have the *dv2* allele in common with Ldn because they showed a single gene difference, *dv1vs Dv1*.

Advanced study of albino

Breeding homozygous lines for each of the two duplicated *abn* genes. The preliminary study showed that F₂ progenies of the cross Ldn x (Vernal x *sv Ne2-4x*) F₁ segregated some albinos together with other chlorophyll abnormalities (Table 1). About ten green F₂ plants (being the *ne2ne2* homozygotes, free of *Ne2*) were selected from these progenies, and self-pollinated. Their F₃ generations were tested for segregation of albinos. F₃ progenies derived from four F₂ plants segregated albinos. A few green sibs were self-pollinated in each of the four F₂ families. F₄ progenies also were tested for albino segre-

gation. Three to five F₄ progenies that segregated green and albino plants at nearly a 3:1 ratio were selected from each F₂ family. Five green sibs were selected in each F₄ progeny, and subsequent progenies (F₅ generation) again tested for albino segregation. Totally, 74 F₅ progenies were tested, of which 32 were fixed for the green phenotype and assumed to be homozygous for one of the duplicated albino genes. Of those progenies, 20 were used in the following allelism test of *abn* genes.

Allelism test of albino genes. Each of the 20 test lines and a normal Ldn line were crossed to at least two other test lines. The F₁ hybrids (in Table 4, hybrids between F₆ lines) were self-pollinated, and their F₂ progenies tested for segregation of albino plants. Table 4 shows that neither the F₂'s of crosses between lines of the No. 1 to 3 F₂ families (Group I cross combinations in the Table) nor those between lines of the No. 4 family (Group II combinations) segregated any albino plants, whereas F₂'s between lines of the No. 1 to 3 families and those of the No. 4 family (Group III combinations) all segregated some albino plants. These findings indicate that lines of the No. 1 to 3 F₂ families carry one of the duplicated albino genes (*abn1*), whereas those of the No. 4 family carry the other one (*abn2*). Ldn should have *abn1*.

Aneuploid analysis of albino genes. Because Ldn is a carrier of the *abn1* gene, the *abn2* gene alone was subjected to aneuploid analysis, for which a few lines of the *abn2* homozygote (Group II lines in Table 4) were crossed

Table 4. Segregation of albinos in self-pollinated progenies of hybrids between F₆ lines derived from the cross Ldn x (Vernal x *sv Ne2-4x*) F₁.

Cross	No. of plants			%	Cross	No. of plants			%
combination	Total	Normal	Albino	albino	combination	Total	Normal	Albino	albino
Group I					Group II				
Ldn (–) x 165 (1)	400	400	0	0.0	183 (4) x 184 (4)	400	400	0	0.0
161 (1) x 165 (1)	381	381	0	0.0	184 (4) x 235 (4)	394	394	0	0.0
157 (1) x 161 (1)	385	385	0	0.0	191 (4) x 242 (4)	400	400	0	0.0
157 (1) x 200 (3)	397	397	0	0.0					
165 (1) x 200 (3)	399	399	0	0.0	Group III				
158 (1) x 208 (3)	396	396	0	0.0	Ldn (–) x 191 (4)	398	382	16	4.0
208 (3) x 179 (2)	398	398	0	0.0	274 (3) x 191 (4)	397	382	15	3.8
179 (2) x 176 (2)	397	397	0	0.0	274 (3) x 242 (4)	392	362	30	7.7
176 (2) x 167 (2)	399	399	0	0.0	151 (1) x 183 (4)	395	382	13	3.3
167 (2) x 171 (2)	401	401	0	0.0	151 (1) x 235 (4)	396	388	8	2.0
171 (2) x 170 (2)	398	398	0	0.0					
170 (2) x 176 (2)	400	400	0	0.0					
158 (1) x 197 (3)	401	401	0	0.0					
197 (3) x 253 (3)	400	400	0	0.0					
253 (3) x 274 (3)	400	400	0	0.0					

Note: Numbers in parentheses indicate F₂ families.

to Ldn DS lines and to a normal Ldn line as the control. The F₁ hybrids were self-pollinated, and the F₂ progenies tested for abn plant segregation.

Table 5 shows that the overall frequency of abn plants was lower than the 6.25% expected based on two recessive gene segregations. Again, the disadvantage for fertilization of having *abn*-carrying pollen as compared to *Abn*-carrying pollen is indicated. Because the female fertility of the disomic F₁'s was normal (ca. 98% after self pollination), the disadvantage for fertilization of having *abn1abn2*-pollen is estimated to be ca. 15%.

To identify which chromosomes carry the *abn* loci, abn frequencies in F₂'s of the DS lines were compared to the disomic ratio. Only a single line, DS 2D(2A), which segregated no albino, deviated significantly from the disomic ratio. This is explained by the hypothesis that chromosome 2A of Ldn carries the *abn1* gene and that the 2D chromosome of CS carries its suppressor. Owing to the homoeology of chromosomes 2A and 2D, this suppressor was named *Abn3* for its presence in the third wheat genome.

The presence of the *Abn3* gene on chromosome 2D should reduce the frequency of abn plants in the F₂ population of the cross DS 2D(2B) x *abn2* homozygote as compared to the disomic frequency, but it did not (ref. Table 5), indicative that chromosome 2B of the *abn2* homozygote carries *abn2*, the other albino gene. Therefore, 2.2% of the above F₂'s would be expected to be albino, assuming that the respective frequencies of the 2D-free female and

male F₁ gametes are 19.7% and 62.5% (after Joppa and Williams, 1988) and that there is a 15% disadvantage for participation in fertilization in having *abn1abn2*-pollen as compared to having wild type pollen. The observed frequency of 3.3% fits this expected frequency ($\chi^2 = 2.81$, df = 1). Two conclusions therefore were drawn: (1) *abn1* and *abn2* respectively are located on chromosomes 2A and 2B, and (2) chromosome 2D of CS carries *Abn3*, the wild-type homoeoallele.

Gene origins of the three chlorophyll abnormalities

The *sv* trait first was found in six-times self-pollinated offspring of the SB₂ hybrids, JF x Vernal³, in which Vernal was the recurrent pollen parent. Of the SB₂ hybrids, those having the normal chromosome complements of Emmer wheat were selected cytologically and used for further self-pollination. Because *sv Ne2-4x* has both the *sv1* and *sv2* genes and Vernal shows a single gene difference, the undoubted origin of either *sv1* or *sv2* is Vernal, and therefore the other gene must be derived from JF. It is not clear, however, which gene is from Vernal and which from JF.

As for the origin of *dv* genes, the F₂ generation of the cross, Vernal x Ldn, segregated some *dv* plants (ref. Table 6), indicative that the *dv* trait is controlled by two recessive genes and that Vernal carries one and Ldn the other. The *dv* homozygote is the double recessive for those genes. One of them, *dv1*, is located on chromosome 2B of the *dv* homozygote, and Ldn carries the dominant

Table 5. Albino frequencies in the F₂ progenies of the crosses Ldn DS's x *abn2* homozygote.

F ₂ line	No. progenies	No. seeds sown	No. seeds germinated	% germination	No. albinos	% albinos	χ^2 -value ¹⁾
1D(1A)	6	600	588	98.0	16	2.72	0.67
2D(2A)	6	600	593	98.8	0	0.00	12.63**
3D(3A)	6	598	578	96.7	7	1.21	1.73
4D(4A)	6	600	595	99.2	14	2.35	0.12
5D(5A)	5	600	583	97.2	14	2.40	0.17
6D(6A)	6	600	591	98.5	15	2.54	0.35
7D(7A)	6	600	582	97.0	10	1.72	0.30
1D(1B)	6	449	440	98.0	15	3.41	2.29
2D(2B)	6	510	489	95.9	16	3.27	2.00
3D(3B)	7	600	586	97.7	7	1.19	1.82
4D(4B)	6	600	596	99.3	19	3.19	1.96
5D(5B)	6	600	579	96.5	8	1.38	1.10
6D(6B)	6	146	143	97.9	3	2.10	0.00
7D(7B)	6	600	581	96.8	13	2.24	0.03
Disomic	6	1,200	1,190	99.2	25	2.10	—
Ldn	5	600	600	100.0	0	0.00	12.78**
<i>abn2</i> homo.	6	600	599	99.8	0	0.00	12.76**
Total ²⁾	—	8,303	8,121	97.8	182	2.24	—

1) Tested against the disomic F₂ ratio2) Total F₂'s except the 2D(2A) and 2D(2B) lines

**: Significant at the 1% level of probability

Table 6. Segregation of three chlorophyll abnormalities in the F₂ generations of the parental stocks.

Material	Greenhouse				Field				
	No. seeds	No.	No.	%	Total	No.	No.	%	%
	sown	germ.	albinos	albinos	grown	sv	dv	sv	dv
Vernal x Ldn F ₂	600	513	7	1.4	516	0	5	0.0	1.0
Vernal x <i>sv Ne2-4x</i> F ₂	320	291	0	0.0	290	63	0	21.7	0.0
<i>sv Ne2-4x</i> x Vernal F ₂	300	262	0	0.0	263	56	0	21.3	0.0
Ldn (control)	430	421	0	0.0	421	0	0	0.0	0.0
<i>sv Ne2-4x</i> (control)	30	24	0	0.0	24	24	0	100.0	0.0

Note: The observed ratio of 553 normal : 119 sv in the (Vernal xx *sv Ne2-4x*) F₂'s fits a 3 : 1 ratio.

allele *Dv1* on the same chromosome. Clearly, the *dv1* and *dv2* of the *dv* homozygote originated respectively from Vernal and Ldn.

The F₂ generation of the same cross, Vernal x Ldn, also segregated some albino plants. Because Ldn carries the *abn1* gene (ref. Table 4), Vernal must carry the other albino gene, *abn2*. The respective origins of *abn1* and *abn2* therefore are Ldn and Vernal.

DISCUSSION

Overall genetic systems for chlorophyll abnormalities. Loci and alleles identified for the three chlorophyll

abnormalities of striato-virescence, delayed virescence, and albino are summarized in Table 7.

Frankel (1950) reported triplicated recessive genes for striato-virescence in common wheat. The present studies indicate the presence of two loci, *sv1* and *sv2*, in Emmer wheat, and a third *sv3* locus in the D genome of common wheat in agreement with his findings. Nishikawa (1986) reported strong association of the albino phenotype with a 2B telosome in various hybrid populations derived from F₁'s between Vernal and 2B double ditelocentrics of the durum wheat 'LD222'. This suggests that the *abn1* gene is present on chromosome 2B of LD222 as in Ldn and of *abn2* in Vernal, which supports

Table 7. Genotypes determined by aneuploid analyses of parental and derivative strains of the three chlorophyll abnormalities.

Strain	Phenotype	Character / chromosome / locus								
		Striato-virescence			Delayed virescence			Albino		
		3A	2A	2D	2A(?)	2B	2D	2A	2B	2D
		<i>sv1</i>	<i>sv2</i>	<i>sv3</i>	<i>dv2</i>	<i>dv1</i>	<i>dv3</i>	<i>abn1</i>	<i>abn2</i>	<i>abn3</i>
Langdon	normal	<i>Sv1</i>	<i>Sv2</i>	–	<i>dv2</i>	<i>Dv1</i>	–	<i>abn1</i>	<i>Abn2</i>	–
Vernal ¹⁾	normal	<i>Sv1</i>	<i>sv2</i>	–	<i>Dv2</i>	<i>dv1</i>	–	<i>Abn1</i>	<i>abn2</i>	–
<i>sv Ne2-4x</i>	sv	<i>sv1</i>	<i>sv2</i>	–	<i>Dv2</i>	<i>dv1</i>	–	<i>Abn1</i>	<i>abn2</i>	–
<i>sv</i> homozygote	sv	<i>sv1</i>	<i>sv2</i>	–	?	<i>Dv1</i>	–	?	<i>Abn2</i>	–
<i>dv</i> homozygote	dv	?	<i>Sv2</i>	–	<i>dv2</i>	<i>dv1</i>	–	?	<i>Abn2</i>	–
<i>abn1</i> homozygote	normal	<i>Sv1</i>	<i>Sv2</i>	–	?	<i>Dv1</i>	–	<i>abn1</i>	<i>Abn2</i>	–
<i>abn2</i> homozygote	normal	<i>Sv1</i>	<i>Sv2</i>	–	?	<i>Dv1</i>	–	<i>Abn1</i>	<i>abn2</i>	–
Chinese Spring	normal	–	–	<i>Sv3</i>	–	–	<i>Dv3</i>	–	–	<i>Abn3</i>

–: No locus, ?: Not identified

1) Alternative gene assignments for Vernal: *sv1* gene on chromosome 3A and *Sv2* gene on chromosome 2A

the present findings.

Except for *sv1*, all the loci are located on group 2 homoeologous chromosomes. Chromosome 2A bears the *sv2*, *dv2* (possibly), and *abn1* loci. Of the three mutants, the *sv2* allele is linked to neither *dv2* nor *abn1*. The last two genes may be linked, but recombination takes place between them because both delayed virescent and albinotic plants were segregated among normal plants in self-pollinated offspring of a single hybrid plant (ref. Table 1). All three mutant genes therefore must have separate loci. Similarly, chromosome 2B carries the *dv1* and *abn2* loci. Again, recombination takes place, providing evidence that their loci differ (Tables 1 and 7).

Two other types of chlorophyll abnormalities, virescence and chlorina, have been reported in common wheat (ref. McIntosh et al., 1998). Virescence is controlled by two loci; *v1* on chromosome 3B (Neatby's virescence) and *v2* on chromosome 3A (Hermsen's virescence). Chlorina is controlled by three homoeoloci; *cn-A1*, *cn-B1* and *cn-D1* on group 7 chromosomes. Here, except for the *sv1* locus on chromosome 3A, all the *sv*, *dv* and *abn* loci located on group 2 chromosomes are independent of the *v* and *cn* loci. Virescence, chlorina, striato-virescence, delayed virescence, and albino therefore all have different genetic bases, but whether there is a relationship between *v2* and *sv1*, both on chromosome 3A, is not known.

Use of the duplicated recessive genes in phylogenetic studies of Emmer wheat. Complementary genes, such as the *Ne1* and *Ne2* for hybrid necrosis, *Ch1* and *Ch2* for Type 1 hybrid chlorosis, and *Cs1* and *Cs2* for Type 2 hybrid chlorosis (Hermsen, 1963; Tsunewaki and Nakai, 1973), have proved useful in studies of phylogenetic differentiation in polyploid wheats. This type of system is extremely efficient because the genotype of the individual being tested can be determined immediately from phenotypes of the F₁ hybrids between the testers and that

individual. Unfortunately, in polyploid wheats the known complementary gene systems are few.

In Emmer wheat, the duplicated recessive genes for chlorophyll abnormalities reported here can be used. For example, Ldn and Vernal can be used as respective testers for the delayed virescence genes *dv1* and *dv2*. If a cultivar carries *dv1* or *dv2*, its F₂ progeny from a cross to Ldn or Vernal will segregate some delayed virescent plants. For albino, the same tester combinations, Vernal (or the *abn2* homozygote bred here) and Ldn (or the *abn1* homozygote) respectively can be used to determine the presence of *abn1* and *abn2* genes. For striato-virescence, we need to breed a tester set, *sv1* and *sv2* homozygotes, from the present *sv* homozygote.

The efficiency of using this type of gene system, i. e., duplicated recessive genes, in phylogenetic studies is much lower than using the complementary gene systems because genotype determination only is possible by observing F₂ segregation which requires additional generation time and a large number of offspring for segregation detection. A presumed advantage is the probable existence of a greater number of duplicated recessive gene systems in polyploid wheats as compared to complementary gene systems. This is suggested by the fact that all three chlorophyll abnormalities studied are controlled by this type of gene system.

Limitation of aneuploid analysis of using DS lines for characters controlled by duplicated recessive genes. In common wheat, monosomic analysis that uses a complete series of 21 monosomics (Sears, 1954), has proved a simple, very effective means of determining the chromosomal locations of genes for a variety of characters. In Emmer wheat, Mochizuki (1968) produced a similar monosomic series. Its use in the gene analysis of Emmer wheat, however, is limited because recovery of monosomics in the offspring averaged only 11% for all the

monosomics compared to the ca. 75% for common wheat offspring. No monosomic analysis therefore has been conducted with the Mochizuki's series. The set of DS lines of Ldn wheat produced by Joppa and Williams (1988) provides an alternative means of aneuploid analysis. Konzak and Joppa (1988) first used the set in their gene analysis of chocolate chaff in durum wheat and successfully located the responsible recessive gene, *cc*, on chromosome 7B of that wheat and its suppressor (wild-type homoeoallele) on 7D of CS. My previous work on Type 2 hybrid chlorosis, in which Ldn DS lines were used, also was successful in locating *Cs1* on chromosome 5A of Emmer wheat and *Cs2* on chromosome 4G of Timopheevi wheat (Tsunewaki, 1992).

In contrast, the present findings on the chromosomal bases of three chlorophyll abnormalities are somewhat ambiguous, because all are controlled by duplicated recessive genes plus their wild-type homoeoalleles present on a D-genome chromosome of CS. As for the striato-virescence (*sv*) controlled by the duplicated loci *sv1* and *sv2*, there is certainty that the location of the *sv1* gene is on chromosome 3A, whereas the location of *sv2* on chromosome 2A was deduced from a higher frequency of *sv* plants in offspring of the 2D(2A) line than in those of the 2D(2B) line, despite the fact that both carry the same suppressor, *Sv3*, on their 2D chromosomes.

Delayed virescence (*dv*) also was shown to be controlled by the duplicated recessive genes *dv1* and *dv2*. The *dv* homozygote and Ldn differ in a single gene pair on their 2B chromosomes; the former has the *dv1* allele, the latter the *Dv1* allele. Direct evidence also indicated that chromosome 2D of CS carries its wild-type homoeoallele, *Dv3*. F_2 data for the cross Ldn x Vernal show that each parent has one duplicated recessive gene, *dv1* or *dv2*. Because Ldn was shown to carry *Dv1* gene, this wheat was deduced as having the *dv2* gene and Vernal *dv1*. The chromosome location of the *dv2* locus is not clear from these findings. Based on the homoeology of chromosomes 2B and 2D, which respectively carry the *dv1* and *dv3* loci, I assumed that the third locus, *dv2*, may be on the remaining group 2 chromosome, 2A.

As for albino (*abn*), both the allelism test results for the *abn* homozygotes and the F_2 data for the cross Ldn x Vernal indicated that this character also is controlled by two recessive genes, *abn1* and *abn2*, and that Ldn carries the former and Vernal the latter gene. Aneuploid analysis of a fixed line for the *abn2* gene showed that the 2A chromosome of that line carries the *abn1* gene and that chromosome 2D of CS carries its wild-type homoeoallele, *Abn3*. Evidence for the *abn2* gene being on chromosome 2B is indirect. F_2 progenies of the DS 2D(2B) line segregated *abn* plants at a high frequency than those of the DS 2D(2A) line, being similar to that of the control F_2 's, although this line carries the suppressor *Abn3* on its chromosome 2D.

All the findings indicate that aneuploid analysis of Emmer wheat done with a set of Ldn DS lines is limited for making a clear-cut determination of the carrier chromosomes of duplicated recessive genes, in particular when a D-genome chromosome carries the wild-type homoeoallele. Taking into account this limitation, at present the set of Ldn DS lines is the only useful means of determining the chromosomal position of genes in this wheat group.

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