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# Cytogenetical studies in wheat

## II. An analysis of chlorophyll abnormalities in hexaploid wheat

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### 1. INTRODUCTION

Spontaneous chlorophyll abnormalities are rare in polyploid wheat, presumably due to double and treble doses of genes controlling each step in normal chlorophyll synthesis. Recessive mutations will not be expressed unless all copies of the gene are in the recessive state. Natarajan, Sikka & Swaminathan (1958) found that treatment with mutagenic agents produced a significantly greater number of chlorophyll aberrations in only one of several hexaploid wheat varieties, indicating that varieties of normal phenotype may differ in the degree of diploidization of genes controlling normal chlorophyll production.

Genetic segregation following hybridization may reveal phenotypically unexpressed recessive mutations when varieties carry mutant alleles at different loci which act in duplicative fashion. For example Froier (1946) and Morrison (1957) cited a number of instances in which chlorophyll-deficient segregants were obtained from intersubspecific tetraploid wheat crosses, and from crosses between tetraploid and hexaploid species. Neatby (1933) also found a viable recessive yellow seedling mutant (Neatby's virescent) in a cross between the varieties Garnet and Double Cross of common wheat (*Triticum aestivum* L. em. Thell. subsp. *vulgare* MacKey). This condition was inherited monofactorially and the gene concerned was located on chromosome 3B (Sears, 1954). A simple dominant chlorophyll mutant resulting from segregation in a cross of multiple ancestry was reported by Jensen (1957).

Nullisomy may also reveal mutant duplicative recessive loci if the chromosome pair which is absent carries loci with normal activity. The present paper reports analyses of albinism in common wheat crosses in which the albinic condition has been associated with nullisomy for chromosome 7B. Presumably 7B nullisomy permitted the expression of a mutant gene (or genes) on (an)other chromosome(s).

In one of the crosses a *viridis* (light green and lethal (von Wettstein, 1961)) type was also found. This was not associated with aneuploidy but was due presumably to a mutation of recent occurrence in the material investigated.

### 2. MATERIALS AND METHODS

Chlorophyll-deficient segregants were obtained in seedling populations derived from monosomic $F_1$ wheat plants. The first instance was in certain $F_3$ lines from the cross Chinese Spring monosomic-7B × Kenya W1483 (W numbers refer to the

Sydney University Wheat Accession Register) where segregation of green, albino and/or chimaeric seedlings (green with longitudinal albino sectors) occurred; in the second instance *albina* and *viridis* segregants were found in $F_2$ progeny of the cross Redman monosomic-7B × Khapstein W1451.

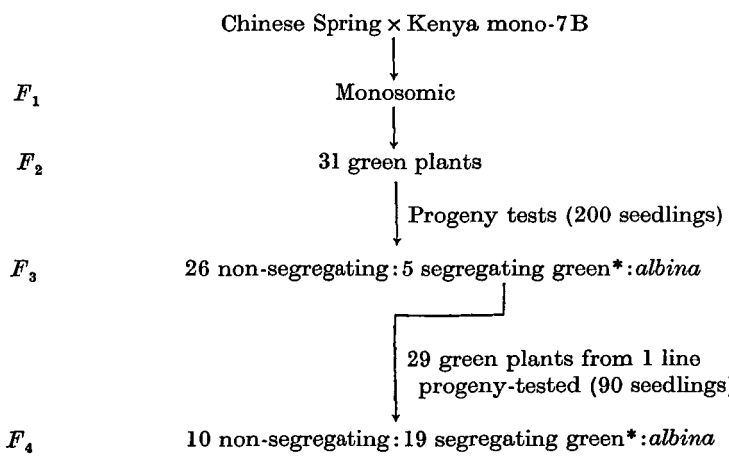
Since progeny originating from monosomic $F_1$ plants involving other chromosomes in both of these series of crosses produced no aberrant seedlings, it was suspected that the chlorophyll deficiencies were associated with chromosome 7B aneuploidy. Cytological and genetical studies were conducted to determine the relationship between chromosome constitution and plant phenotype. The procedure was to follow the transmission of chromosome and chlorophyll abnormalities in pedigree lines.

For somatic chromosome counts, seedling root-tips were pretreated in cold water, fixed in acetic-alcohol and stained by the Feulgen procedure. Meiosis was studied in pollen mother cells from selected anthers similarly fixed and stained.

## 3. RESULTS

### (i) *Chinese Spring monosomic-7B × Kenya*

Ten chimaeras (0·8 %) and 8 albinos (0·8 %) were observed among 1210 seedlings from 5 $F_3$ lines from a monosomic $F_1$ plant (Text-fig. 1). Twenty-nine green plants from one of the segregating lines were progeny-tested. Segregation ratios in




Text-fig. 1. Pedigree and segregation patterns for chlorophyll phenotypes in cross Chinese Spring mono-7B × Kenya. * Including chimaeric seedlings.

samples of approximately 90 seedlings were recorded and somatic chromosome numbers determined on a maximum of 5 seedlings in each progeny. Chromosome counts indicated that 19 of the $F_3$ progeny-tested plants were monosomic and 10 disomic. Each of the 19 monosomics segregated green and albino seedlings and, in most instances, included a low proportion of chimaeras, whereas the 10 disomics were true breeding for normal green phenotype. Eighty-three (5·0 %) seedlings of

1648 scored in the monosomic progenies were albino and 19 were chimaeric (1·2 %). Further cytological studies on $F_4$ seedlings showed that albino plants invariably had 40 chromosomes and green plants usually possessed 41 or 42 normal chromosomes. A low frequency of green plants with 41 or 42 chromosomes but including one or more telocentrics was also noted.

At meiosis, the 41-chromosome green plants exhibited 20 bivalents and a univalent. Green tissue in 6 chimaeric plants was studied meiotically; 4 plants possessed 20 bivalents and an unpaired telocentric, and 2 had 20 bivalents and an isochromosome. Presumably albino sectors in each plant resulted from loss of the telocentric or isochromosome during somatic development.

Results presented above show that in the $F_3$ line selected for investigation, chromsome 7B possessed a gene (or genes) necessary for the production of normal green phenotype since only nulli-7B seedlings were albino. Furthermore, studies on chimaeric plants indicated that the presence of one arm of chromosome 7B was sufficient to ensure the normal phenotype. Since chromosome 7B would have been monosomic in each generation, the source of the monosome in the $F_3$ family and $F_4$ lines was Kenya 7B.

Chinese Spring plants nullisomic for chromosome 7B are green, indicating the presence on other chromosomes of a gene (or genes) capable of duplicating the indicated control of chlorophyll synthesis on 7B. Since other Chinese Spring × Kenya crosses, each disomic for 7B, failed to segregate for albinism, it is apparent that both Chinese Spring and Kenya have normal genes for control of chlorophyll synthesis on this chromosome.

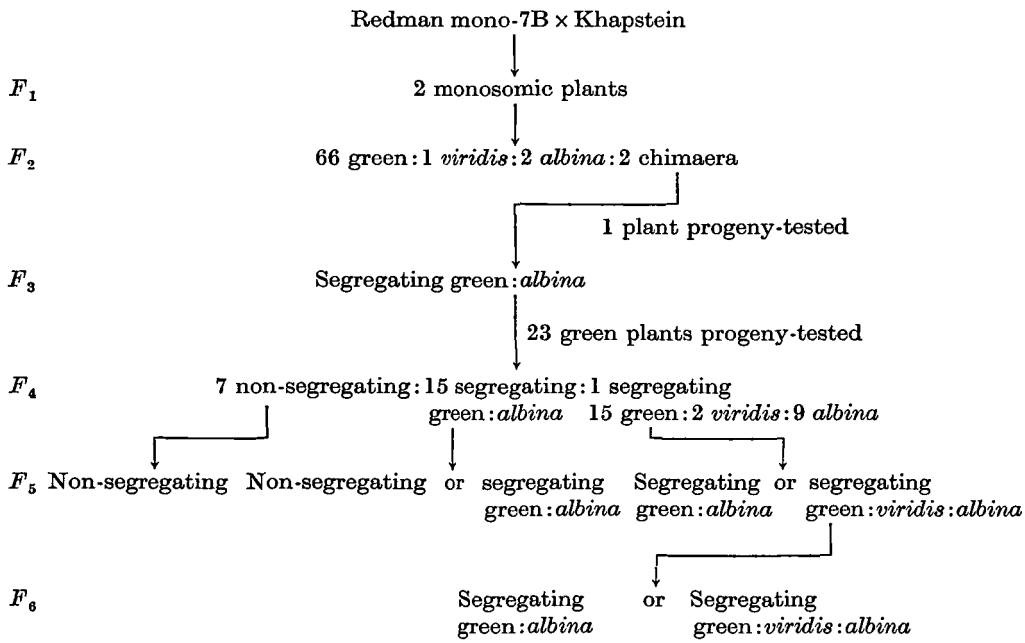
In the $F_4$ lines studied, all 40-chromosome plants were albino and a nullisomic frequency of 5 % was obtained. However, of the 31 $F_2$ plants progeny-tested, only 5 segregated, with an over-all frequency of 0·8 % albinos within segregating lines. The lower frequency in $F_3$ lines implies segregation for genes determining albinism on chromosomes other than 7B. From the breeding behaviour of wheat monosomics it is expected that about three-quarters (approximately 24) of the 31 $F_3$ lines would be derived from monosomic $F_2$ individuals. If the genotypes of Chinese Spring and Kenya on chromosomes other than 7B differed by a single gene relative to the system under investigation, then three-quarters of the lines (or 18) derived from $F_2$ monosomic plants should have produced albino segregants. The observed proportion (5 in 31) of $F_3$ lines segregating in this manner suggests that appearance of albino seedlings depended on segregation at more than one locus on other chromosomes in the residual genome.

### (ii) *Redman monosomic-7B × Khapstein*

A summary of results in relation to generation sequence is given in Text-fig. 2. the progenies of two monosomic $F_1$ plants 66 green, 1 *viridis*, 2 *albina* and 2 green/*albina* chimaeric seedlings were recorded. Subsequent studies were confined to populations derived from one of the chimaeras. Progeny tests on 23 green $F_3$ plants showed that 7 were true-breeding, 15 segregated green and *albina* seedlings (including a low proportion of chimaeras), and one segregated 15 green : 2 *viridis* : 9 *albina*.

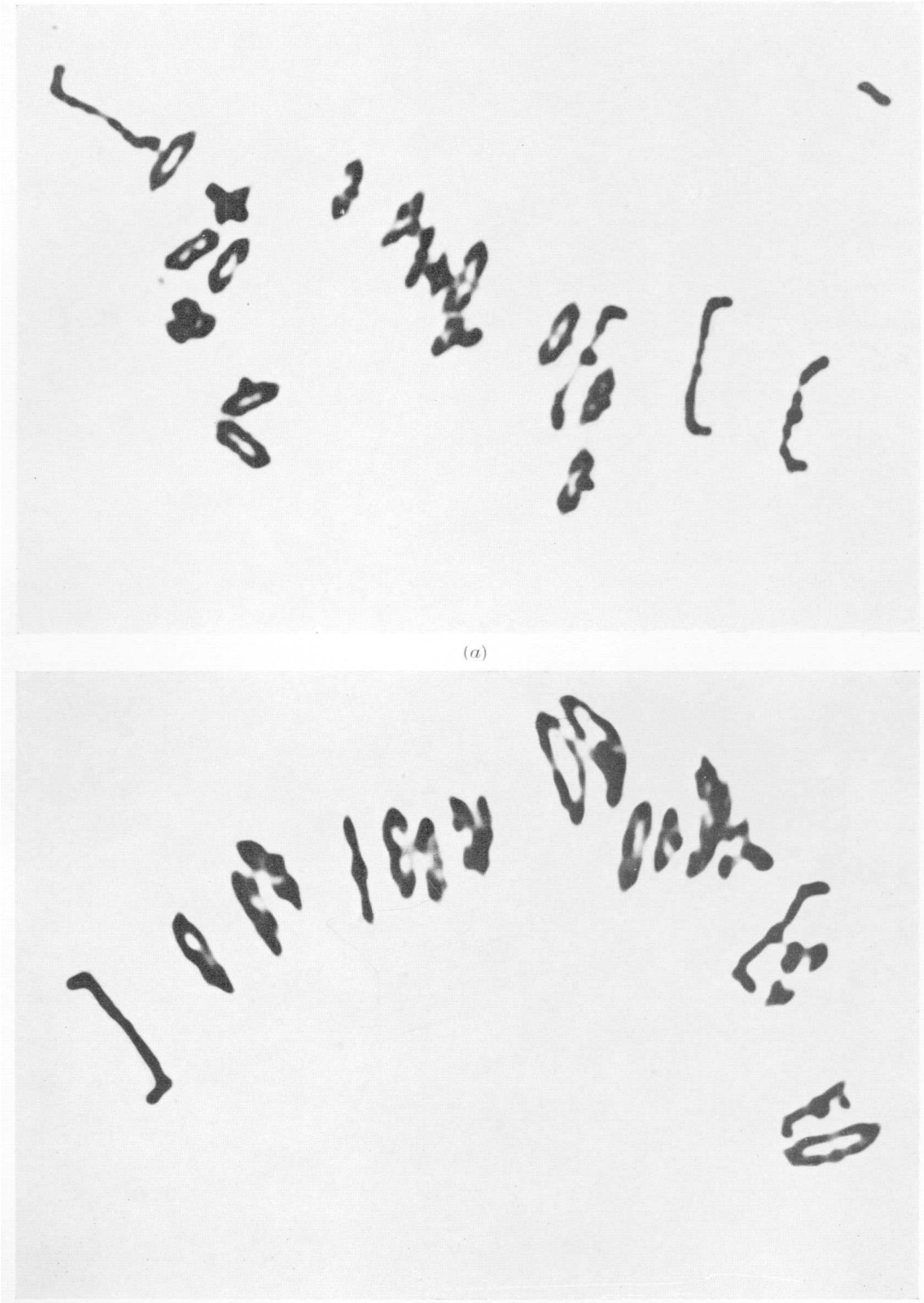
The results for the line producing *viridis* segregants are deferred since it was found, subsequently, that certain derivatives of this line differed in chromosome constitution from the other 22 lines.

The 7 pure-breeding green families in $F_4$ were again true-breeding in $F_5$. Green plants from the 15 segregating families were pure-breeding or segregated green (including chimaeric) and albino seedlings. Somatic chromosome counts revealed that all individuals examined in pure-breeding progenies possessed 42 chromosomes, including 2 large telocentrics. Green plants in segregating progenies had either 41 chromosomes, including a large telocentric, or 42 chromosomes, including 2 telocentrics. Albino seedlings invariably possessed 40 non-telocentric chromosomes.




Text-fig. 2. Pedigree and segregation for chlorophyll phenotypes in cross Redman mono-7B × Khapstein.

Meiotic studies showed that 42-chromosome plants possessed 21 bivalents, one of which was ditelocentric, whereas 41-chromosome plants had 20 normal bivalents and an unpaired telocentric. Presumably albino plants were nullisomic. Albinism was thus prevented by genetic activity of the telocentric chromosome. As with Chinese Spring, normal chlorophyll development occurs in Redman when both 7B chromosomes are absent. Albinism, therefore, resulted from segregation of genes on chromosomes other than 7B and was expressed only in nullisomics, where the duplicative control of chlorophyll production by chromosome 7B had been removed. In order to confirm that the telocentric involved an arm of chromosome 7B and to establish its identity relative to a known telocentric ($7B^L$) in Chinese Spring, $F_5$ plants possessing 20 bivalents and a telocentric were crossed recipro-



(*a*)

(*b*)

Metaphase I configurations in $F_1$ hybrids from a cross between Redman mono-7B × Khapstein monotelosomic derivative and Chinese Spring ditelosomic 7B$^L$. (*a*) Twenty IIs plus telosome. (*b*) Twenty-one IIs including ditelosomic pair (fifth from left). × 2600.

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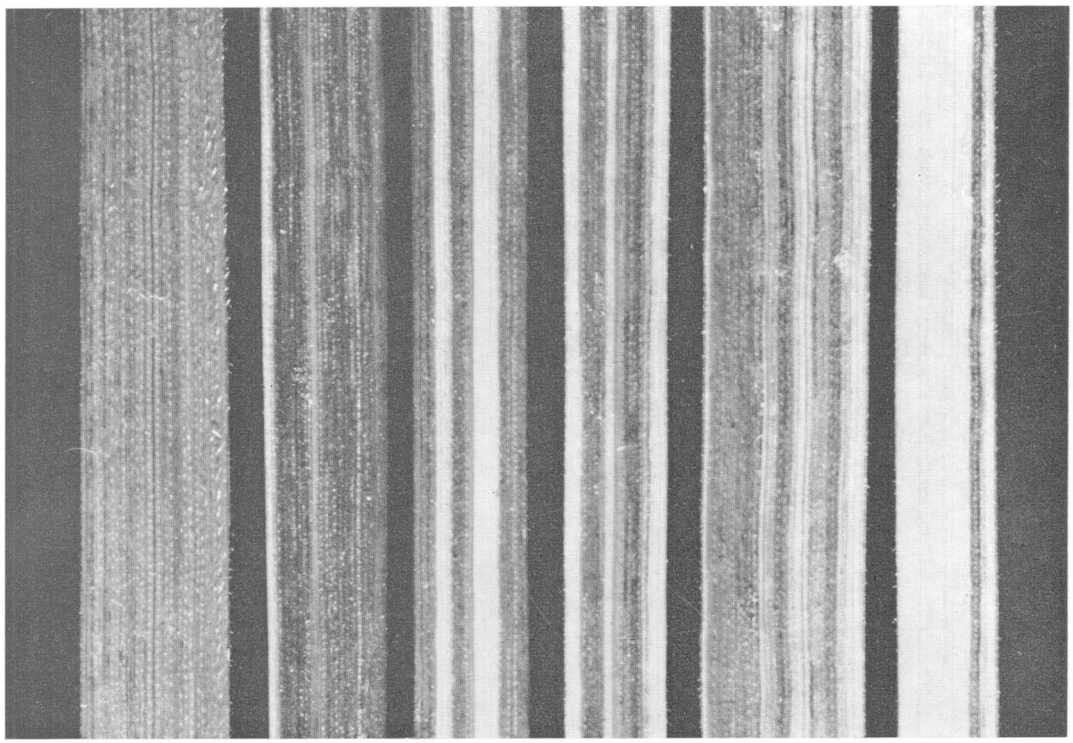
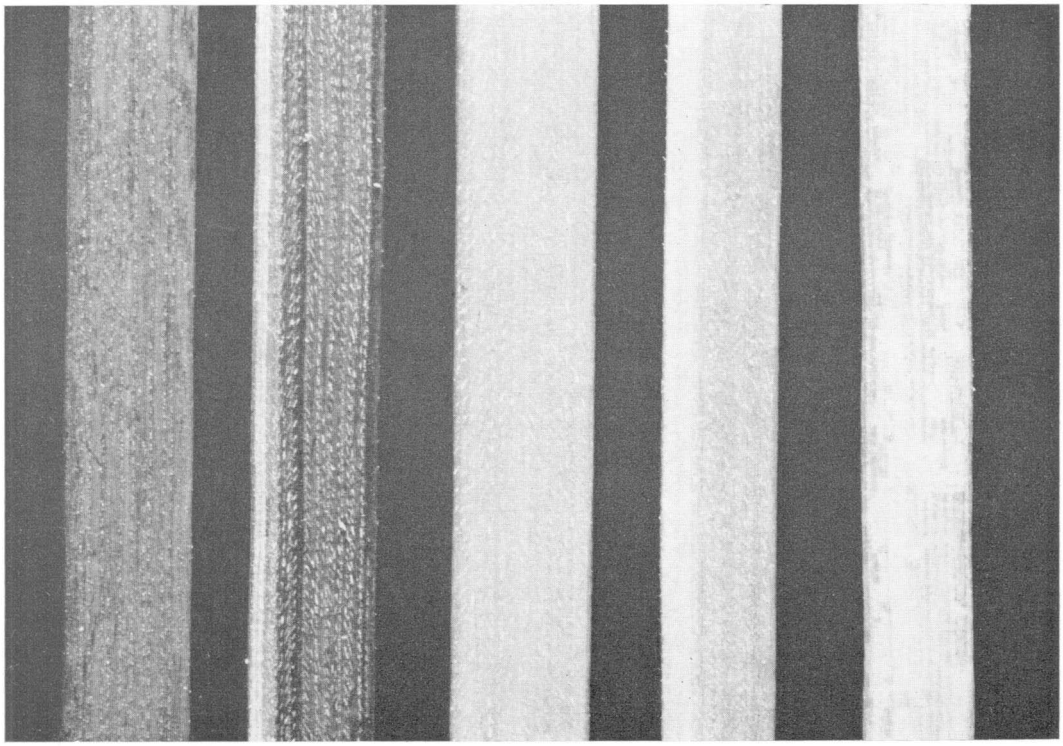


(*a*)



(*b*)

Chimaeric patterns. (*a*) Variation in the number of stripes and extent of albinic sectoring on leaves; left, fully green. (*b*) From left: fully green, *albina*/green, *viridis*, *albina*/*viridis*, *albina*. × 3.

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cally with a Chinese Spring monosomic-7B$^{L}$ plant. Doubly telosomic plants from these crosses possessed 21 pairs including a ditelosomic bivalent (Plate 1), thus confirming that the telocentric implicated was 7B$^{L}$. Monotelosomic $F_1$ plants from the crosses segregated *albina* seedlings in $F_2$ although the proportion of albinos was considerably lower than in the parental material. Of 238 $F_2$ plants 228 were green, 1 chimaeric and 9 albino (3·8%), in contrast to an albino frequency of 30·0% in the parental material. Somatic chromosome counts made on a further 49 randomly selected seedlings detected 11 seedlings with 40 chromosomes. Three of the latter seedlings were albino.

Segregation within the nullisomic population provided genetic confirmation that Chinese Spring chromosomes other than 7B can duplicate the control of chlorophyll-synthesizing activity exercised by 7B and, although the nullisomic sample was extremely small, the segregation ratio was best explained on the basis of a single gene difference.

### (iii) *Exceptional progenies segregating* viridis *seedlings*

From the $F_4$ family segregating *viridis* as well as green and *albina* seedlings, six green plants were progeny-tested. The results are presented in Table 1.

Table 1. *Seedling chlorophyll phenotypes in progenies of green plants from* $F_3$ *line segregating green,* viridis *and* albina

| Plant designation no. | Green | Chimaeric green/*albina* | *Viridis* | Chimaeric *viridis/albina* | *Albina* |
|---|---|---|---|---|---|
| 1 | 135 | 4 | 14 | 2 | 51 |
| 2 | 104 | 4 | — | — | 14 |
| 3 | 138 | 3 | 52 | — | 28 |
| 4 | 70 | — | 5 | — | — |
| 5 | 112 | — | 18 | — | 28 |
| 6 | 45 | 3 | — | — | 6 |

Table 2. *Seedling chlorophyll phenotypes in progenies of* 13 *plants from* $F_5$ *line segregating green,* viridis and albina

| No. of families | Green | Chimaeric green/*albina* | *Viridis* | Chimaeric *albina/viridis* | *Albina* | Total |
|---|---|---|---|---|---|---|
| 9 | 252 | 13 | 107 | 2 | 100 | |
| | 265 | | 109 | | | 474 |
| 4 | 112 | 4 | — | — | 53 | |
| | 116 | | | | | 169 |

Among the progeny of plant no. 1, 2 *viridis/albina*, in addition to 4 green/*albina*, chimaeras were observed. Because plant no. 3 produced a high proportion of *viridis* segregants, further progeny tests were conducted on 13 green individuals

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(Table 2). Nine segregated green, *viridis* and *albina*, and included chimaeras, 2 of which were *viridis*/*albina* types (Plate 2). The remaining 4 progenies segregated green and albino seedlings.

Somatic chromosome counts on $F_6$ seedlings demonstrated that albinos had 40 chromosomes as before; however, green and *viridis* segregants possessed either 41 or 42 non-telocentric chromosomes. At flowering, green plants with 41 chromosomes were usually vigorous and multi-tillered, whereas those with 42 chromosomes were weak with only one or two spike-bearing tillers. Examination at meiosis showed that the former possessed 20 bivalents and an isochromosome, and the only plant examined from the weaker group was di-isosomic. None of the weaker plants set more than 12 seeds, and all were thus considered unsatisfactory for progeny-testing. Sears (1954) noted a similar lack of vigour shown by Chinese Spring plants di-isosomic for $7B^L$. At meiosis 5 green/*albina* chimaeras possessed 20 bivalents and a telocentric, and one was monosomic for an isochromosome.

The absence of pure-breeding green families in these progenies (Table 2) was probably due to preferential selection of monoisosomic plants for study. The *viridis* phenotype was independent of the behaviour of the isochromosome, indicating that in the presence of the normal genetic activity of 7B there was segregation for additional genes affecting chlorophyll synthesis. The 265 green : 109 *viridis* ratio in Table 2 conforms to single gene segregation ($\chi^2$ 3:1 = 3·43; $P$ = 0·10–0·05). On the basis of these results an inspection of Table 1 suggests that in plants nos. 1 and 4 *viridis* seedlings resulted from segregation at duplicate loci, whereas in plant no. 3 *viridis* individuals resulted from segregation at a single locus. However, the results for plant no. 5 conform statistically to neither a single nor a duplicate gene segregation. As plant no. 4 did not give albino offspring, it is probable that it was ditelosomic, although no cytological studies were made.

## 4. DISCUSSION

No instance of chlorophyll-aberrant common wheat due to nullisomy has been reported previously. This suggests that in varieties where nullisomics have been studied the residual genotypes are capable of duplicating any chlorophyll-producing activity controlled by the chromosome pair for which the nullisomic is deficient. The present results show that albino nullisomics occur in the progeny of certain monosomic $F_1$ plants and that their appearance probably follows segregation at loci in the residual genotype. Nullisomy reveals the mutant nature of duplicated loci on other chromosomes. In two unrelated crosses involving different monosomic-7B stocks the albino segregants were nullisomic, indicating that chromosome 7B possesses a gene(s) concerned with normal chlorophyll synthesis. This gene(s) was localised to the long arm ($7B^L$).

The frequency of albinos varied in the two crosses, being dependent upon the morphology, and hence transmission rate, of the 7B chromosome; in Chinese Spring × Kenya, 7B was normal, whereas in Redman × Khapstein a telocentric or an isochromosome was involved. An albino frequency of 5 % was obtained when

7B was normal, whereas with the 7B telocentric the corresponding frequency was 30%. Sears (1954) reported a nullisomic frequency of 1·4% in progenies of Chinese Spring monosomic-7B plants. The much higher frequency of nullisomic progeny from monosomic $7B^L$ plants indicates a high transmission rate of 20-chromosome in competition with 20 + telo-chromosome pollen.

Chimaeric plants from Chinese Spring × Kenya were monosomic for a telocentric or an isochromosome, both of which presumably were somatically unstable. In Redman × Khapstein all lines were derived from a single $F_2$ chimaera and consequently possessed telocentrics or isochromosomes.

When monotelosomic sib-plants from Redman × Khapstein were crossed with Chinese Spring mono-$7B^L$, nullisomic $F_2$ offspring from monotelosomic $F_1$ plants segregated for green and albino phenotypes. The populations available for study were small but the results suggested that Chinese Spring differed from the other parents by a single dominant gene on a chromosome other than 7B.

The present work has similarities with studies in hexaploid oats (*Avena sativa* L.), where a number of chlorophyll abnormalities have been associated with aneuploidy (Philp, 1935; McGinnis & Taylor, 1961; McGinnis & Andrews, 1962; Hacker, 1966). Furthermore, McGinnis & Andrews (1962) and Hacker (1966) presented evidence for duplicative gene activity in chlorophyll synthesis as segregation was shown within nullisomic progenies. Hacker (1966) accounted for chimaeras by suggesting somatic elimination of telocentrics derived from a normal monosome.

Sears (1957) established that the expression of Neatby's virescent gene on chromosome 3B could be modified by genes on the homoeologous chromosomes 3A and 3D. The comparative rarity of chlorophyll mutants in hexaploid wheat might be due to duplication of genes at corresponding loci on homoeologous chromosomes. If so, the duplicative genes affecting chlorophyll synthesis in the current studies may be located on chromosomes 7A or 7D, or both. In these circumstances mutant genes on 7A and/or 7D would permit the expression of albinism in nullisomic 7B plants, and segregation within nullisomic classes would indicate heterozygosity of genes on one or both chromosome pairs.

The *viridis* phenotype was not associated with aneuploidy. The occurrence of occasional *viridis*/*albina* chimaeras indicated that somatic loss of $7B^L$ from potentially *viridis* plants also resulted in albinism and that normal genetic activity of $7B^L$ was essential for *viridis* expression.

The separation of lines yielding *viridis* segregates from those producing albinos was not possible in the material studied in detail as an isochromosome was involved and the presence of two isochromosomes resulted in a marked reduction in plant vigour. Green/*albina* chimaeras in lines producing *viridis* segregants frequently possessed a telocentric derived from the isochromosome. Pedigree selection from such plants should yield lines not producing albinos but segregating *viridis* and green seedlings.

In a series of Redman monosomics × Khapstein crosses *viridis* segregants occurred only in the cross involving monosomic-7B. Since the *viridis* phenotype was not associated with 7B aneuploidy, its presence in only one instance suggests

that it arose through gene mutation, either in the $F_1$ plant itself, or in one of the gametes involved in production of the $F_1$ plant.

Steinitz-Sears (1966) reported different degrees of somatic instability of chromosome-3B$^S$ telocentrics as reflected by dosage expression of Neatby's virescent gene. Somatic loss, or gain, of the telocentrics occurred at different stages in plant development and continued throughout growth. In the current studies chimaerism, when present, was shown always by the primary seedling leaf. Sectoring at later stages probably reflected ontogenetic development from the initial loss.

In the case of barley, which is diploid, Gustafsson (1956) indicated that numerous non-allelic mutations result in abnormal chlorophyll phenotypes. This implies that many single gene-controlled steps are involved in normal chlorophyll biosynthesis. Although the present studies show that albinism in two hexaploid wheat crosses is associated with nullisomy for chromosome 7B, the same gene-controlled step may not be involved in each instance. Further studies on aneuploid populations undoubtedly will reveal the presence of unexpressed mutations and the chromosome location of duplicative genes with normal chlorophyll-synthetic activity.

## SUMMARY

Following segregation in two unrelated wheat crosses, albinism was associated with chromosome 7B nullisomy which permitted expression of mutant genes on chromosomes other than 7B. The frequencies of albino segregants differed in the two instances, being dependent upon the morphology of the 7B monosome, which was either telocentric 7B$^L$ or normal.

A *viridis* phenotype also segregated from one cross. Its expression was independent of 7B aneuploidy. Failure to obtain lines segregating only for green and *viridis* phenotypes was due to semi-lethality resulting from the presence of two 7B isochromosomes.

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