

MONOSOMIC ANALYSIS OF A CHLOROPHYLL DEFICIENCY

IN COMMON WHEAT

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ABSTRACT

The inheritance of albinism in a Triticum durum line from the cross C.I. 8155 x Mindum⁴ was shown to be monogenic and recessive. Both green and albino seedlings were shown to have 28 chromosomes.

This character was transferred to hexaploid wheat by backcrossing to the variety Rescue and selecting segregating progeny after each backcross.

In this material it was found that albino seedlings had 40 chromosomes and green seedlings had from 40 to 42 chromosomes. Albino plants were found to be nullisomic for chromosome 3D which indicates that this chromosome carries a gene for chlorophyll production in the variety Rescue.

The study of F₂ lines from crosses between the hexaploid segregating lines and the Rescue monosomic series failed to reveal a critical chromosome in the A or B genome. There was some evidence however that homoeologous group III is probably involved.

Crosses between Vernal emmer and Marquis failed to produce albino offspring as reported by other authors.

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INTRODUCTION

During the past decade much progress has been achieved in the field of plant aneuploidy. Apart from the value of aneuploids as a genetic tool, their use in practical plant breeding is being explored. The production of aneuploid series, especially monosomics, in polyploid species of economic crops, has resulted in major advances in simplifying the analysis of polyploid inheritance, in locating genes on specific chromosomes and in analyzing chromosome translocations.

Abnormalities in the pattern of chlorophyll expression are not uncommon in the cereal grains and have been studied extensively, particularly those occurring as a result of mutation. In contrast to the number of chlorophyll mutations obtained in barley, chlorophyll mutations in hexaploid wheat are rare. Stadler (17) and others have concluded that radioinsensitivity of the chlorophyll system of hexaploid wheat is primarily due to gene duplication.

Although chlorophyll deficiencies affect populations adversely, they are very useful to the geneticist. They supply easily identifiable loci for chromosome mapping and make excellent markers for studying the effects of various mutagenic agents on mutation frequency in many plants. Chlorophyll-deficient seedling characters may be used to identify and characterize a population. They also enable the geneticist to make inferences relative to types of

gene action and the selective advantage of homozygotes versus heterozygotes.

Philp (5) pointed out that polymeric genes (V genes) are responsible for chlorophyll production and that at least two and probably three loci are involved in chlorophyll production in Avena sativa. McGinnis and associates (8, 9, and 10) have located two genes, V₁ and V₂ for chlorophyll production on chromosomes 15 and 21 respectively, through karyotype analysis and aneuploid studies in Avena sativa.

The object of the present project was to study the inheritance of albinism in a strain of Triticum durum and to determine by means of monosomic analysis the chromosomes involved in the control of this character when it is transferred to hexaploid wheat.

LITERATURE REVIEW

I. CHLOROPHYLL DEFICIENCIES IN OATS

The first reports of albinism in common oats were by Nilsson-Ehle in 1913 and by Akerman in 1922. The latter observing them in hybrids of a Swedish black-hulled variety and the variety Probsteier (reported by Froier (3)).

Philp (5) studying the cytogenetics of albino oats found that albino seedlings had only 40 chromosomes, whereas the parent plants of albinos were monosomic.

McGinnis and associates (8, 9, and 10) have identified chromosomes 15 and 21 of Avena sativa as having genes for chlorophyll production and inferred that a third locus is probably involved since Avena sativa is an allohexaploid.

II. CHLOROPHYLL-DEFICIENCIES IN WHEAT

Froier (3) reported that Meister (1922) obtained albinos from a cross of Triticum durum x T. vulgare and that Vavilov and Jakushkina (1925) found that chlorophyll-deficient plants were produced from crosses between T. vulgare v. lutescens Al. and T. dicoccum v. pycnurum. They also obtained only albino F₁ seedlings from the cross T. durum v. melanopus Al. x T. spelta No.25.

Harrington and Smith (4) reported that in the F₂ generation of a cross between Khapli and Early Emmer, approximately one-sixteenth of the seedlings were bright

yellow in color. When grown in the greenhouse, the yellow seedlings developed some chlorophyll. The results showed that two recessive genes, designated a and b, for the inhibition of chlorophyll development are carried by Khapli and Emmer respectively. When these genes are present in the homozygous condition, the seedlings are yellow.

According to Froier (3), Khintshuk (1929) examined the chlorophyll mutants, albina, xantha and flavescens, in the F₃ and F₄ generations from the cross of Triticum timopheevi Zhuk. var. viticulosum and T. persicum Vav. and suggested that two factors were responsible for chlorophyll production.

Smith and Harrington (16) reported that in the F₂ generation of a cross between Vernal emmer (C.I. 3686) (T. dicoccum) and Marquis (T. vulgare) approximately one sixty-fourth of the seedlings were albinos. They suggested that one parent carries two recessive genes for albinism which are complementary to a third gene carried by the other parent. These three genes when present together in the homozygous recessive condition inhibit the development in chlorophyll.

Froier (3) cited that L. A. Sapehin (1932) reported two factor pairs were responsible for albinism and two factor pairs were responsible for yellow seedlings in T. durum crosses. From crosses between T. durum and

T. dicoccum, he concluded that three factor pairs controlled the albinism.

A mutable recessive gene that causes a virescent condition in Triticum aestivum was reported by Neatby in 1933 (11).

Froier (3) reported that Kihara (1937) found chlorophyll mutants from hybrids of Triticum persicum v. stramineum and T. timopheevi. He concluded that yellow seedlings (xantha or lutescens) were homozygous for one recessive gene y. Seedlings with two recessive genes, a₁ and a₂ were albinos.

Chromosomes of homoeologous group III of common wheat have been shown by Sears (14) to affect chlorophyll formation. Steinitz-Sears (18) found that the gene v (virescent) is located on the short arm of chromosome 3B.

III. PECULIARITIES OF MONOSOMIC OR NULLISOMIC 3D LINES OF COMMON WHEAT

A line of wheat nullisomic for chromosome 3D (XVI) was discovered in the F₅ generation of the pentaploid hybrid Rescue x Golden Ball by Larson (6). A study of chlorophyll turnover and of the products of photosynthesis by means of radioactive tracers in this nullisomic 3D wheat was investigated by Larson and Perkins (7). They found that the nullisomic had a ratio of labelled carotenes to xanthophylls different from that of the other varieties,

and it resembled Golden Ball in having a high amount of C^{14} in the hot water-soluble extract. They suggested that the differences between the nullisomic and the two hexaploid wheats were probably due to the loss of chromosome 3D (7).

Bhowal (1) found an unusual segregation of disomics, monosomics, and nullisomics in the offspring of a substitution of Rescue x Golden Ball. Pollen with 20 and 21 chromosomes occurred in the frequency of 64 and 36%, respectively. The 61% transmission of the deficiency on the male side and 76% on the female explained the unusual segregation in the summer crop. Bhowal (2) also found that normal pollen with chromosome 3D from Red Bobs has little competitive advantage over pollen deficient for chromosome 3D, when a substituted monosomic line of wheat derived from the hybrid Rescue x Golden Ball was selfed.

MATERIALS AND METHODS

The material utilized included:

(I) Lethal Durum - This is a line obtained from an F_3 plant of the cross C.I. 8155 x Mindum⁴ which segregated to produce albino seedlings (shown in Plate I, Figure 1). This stock was produced and supplied by Dr. E. R. Kerber, C.D.A., Winnipeg.

(II) Rescue³ Lethal Durum (R^3LD) is a segregating line derived from crossing Rescue (Triticum aestivum) to Lethal Durum and backcrossing twice to Rescue. Following the second backcross 26 of 41 F_1 plants produced segregating F_2 progeny. These 26 lines were utilized in this study.

(III) The Rescue monosomic series produced by Dr. R. I. Larson, C.D.A., Lethbridge.

(IV) The T. aestivum variety Marquis and the emmer variety Vernal.

Three segregating F_5 lines of Lethal Durum were studied to check the assumption that a single recessive factor conditioned albinism. F_6 lines derived from single F_5 plants were also grown and examined.

Study of the 26 segregating R^3LD lines revealed differences in their segregation ratios and on this basis four with a high albino frequency and three with a low frequency were selected. Six to eight plants from each of the seven lines were cloned. One clone of each plant was grown in the greenhouse and was selfed. The remaining clones of

each plant were maintained in a cold room at 38-42°F. The selfed seeds from the greenhouse grown clones were germinated and the segregation ratio in each progeny recorded. The remaining clones of plants which the progeny test showed to be segregating were used as pollen parents to cross with the 21 Rescue monosomic lines, the high and low frequency groups being used as separate bulk pollen sources. Root tips were collected from all plants in segregating progenies and chromosome numbers determined at mitotic metaphase using the Feulgen method.

Due to the fact that albino seedlings had 40 chromosomes and that green seedlings varied from 40 to 42 chromosomes, monosomic R³LD plants from the lines with both high and low albino frequencies were crossed to normal Rescue to determine the n-1 male gamete transmission rate.

The F₁ seeds from the crosses of the Rescue monosomics x R³LD were germinated and chromosome numbers were determined for all plants at mitotic metaphase. Four hundred and eighty-one F₁ seedlings of Rescue monosomics x R³LD high frequency lines and 498 F₁ seedlings of Rescue monosomics x R³LD low frequency lines were grown and checked for chromosome number.

Spikes from F₁ plants with 40 or 39 chromosomes were collected and fixed in Carnoy's Fluid (6:3:1, alcohol,

chloroform and acetic acid) and analysed at metaphase I of meiosis. All F_2 seeds from such plants were germinated and the segregation ratios and chromosome numbers determined. The F_2 seeds from 70 F_1 plants of Rescue monosomics x R^3LD with 42 chromosomes were likewise analysed.

In an attempt to identify the chromosome involved in the A or B genome, 15 green F_2 plants with 39 or 40 chromosomes from double monosomics (4A, 3D) and (3B, 3D) and seven nullisomic 3D plants from selfed monosomic R^3LD were planted in the greenhouse. The nullisomic 3D plants were used as both female and male parents to cross with F_2 plants from double monosomics (4A, 3D) and (3B, 3D).

Reciprocal crosses of Marquis x Vernal emmer were made. The root tips of each F_1 hybrid were collected and counted. Some F_1 plants were selfed and the remainder were backcrossed to Marquis. The root tips of F_2 and backcrossed F_1 seedlings were collected. F_3 seeds and a large sample of backcrossed F_2 seeds were germinated in the germination boxes to examine the color of seedlings.

RESULTS AND DISCUSSION

I. THE MODE OF INHERITANCE IN LETHAL DURUM

The segregation ratios for the three F₅ lines are included in Table 1. All approximated a ratio of 3 green to 1 albino as do the pooled results. This would indicate that albinism is monogenically controlled.

The green plants of each F₅ line were selfed to produce seed for F₆ families. It was expected that 1/3 of the families would breed true and 2/3 segregate. The data in Table 2 agrees with the expected frequencies.

Table 3 summarizes the segregation occurring in each segregating F₆ family. All fit the theoretical expectation but a significant homogeneity χ^2 is attained for family 1. On the basis of all the data, however, it is apparent that the occurrence of albino seedlings in the Lethal Durum strain is simply inherited and due to the homozygous recessive condition.

A total of 89 green and 21 albino seedlings were cytologically examined and all had a 2n number of 28.

II. RESCUE³ LETHAL DURUM

The segregation in each of the 26 R³LD F₂ lines is presented in Table 4. Those selected as the low frequency group included 1-2, 1-3, and 1-6 while the high frequency group included 1-9, 3-12, 3-13 (progeny test showed high

TABLE 1.
 SEGREGATION IN THREE LETHAL DURUM F₅ LINES

Lethal Durum F ₅ Line	Number of Seedlings		X ² -Value (3:1)	Probability
	Green	Albino		
No.1	21	4	1.08	0.25-0.50
No.2	17	8	0.65	0.25-0.50
No.3	15	8	1.17	0.25-0.50
Totals	53	20	0.22	0.50-0.75

TABLE 2.
THE RATIO OF SEGREGATING AND NON-SEGREGATING
LINES IN LETHAL DURUM F₆ FAMILIES.

Lethal Durum F ₆ Family	Observed		Expected (1:2)		X ² -Value (1:2)	Probability
	Non-segregating Lines	Segregating Lines	Non-segregating Lines	Segregating Lines		
1	4	8	4	8	0	1
2	7	9	5.33	10.67	0.78	0.25-0.50
3	7	10	5.67	11.33	0.47	0.25-0.50

TABLE 3

SEGREGATION RATIOS IN LETHAL DURUM F₆ FAMILIES

Line	Number of Seedlings			X ² - Value (3:1)	Probability
	Green	Albino	Total		
1- 2	17	1	18	2.6667*	0.10-0.25
1- 3	7	5	12	1.7778	0.10-0.25
1- 4	11	7	18	1.8519	0.10-0.25
1- 5	4	5	9	3.0000*	0.05-0.10
1- 6	6	1	7	0.4286	0.50-0.75
1- 8	9	2	11	0.2727	0.50-0.75
1- 9	14	1	15	1.8000*	0.10-0.25
1-10	15	2	17	0.9608*	0.25-0.50
Totals	83	24	107		
2- 2	17	7	24	0.2222	0.50-0.75
2- 3	18	6	24	0	1
2- 4	15	5	20	0	1
2- 7	14	1	15	1.8000*	0.10-0.25
2- 8	16	8	24	0.8889	0.25-0.50
2-10	11	3	14	0.0952	0.75-0.90
2-12	18	5	23	0.1304	0.50-0.75
2-14	12	4	16	0	1
2-18	11	3	14	0.0952	0.75-0.90
Totals	132	42	174	4.1208(9df) 0.0690(1df)	
3- 1	17	6	23	0.0145	0.90
3- 3	19	11	30	2.1778	0.10-0.25
3- 5	16	3	19	0.8305	0.25-0.50
3- 8	10	3	13	0.0256	0.75-0.90
3-10	19	5	24	0.2222	0.50-0.75
3-11	11	5	16	0.3333	0.50-0.75
3-14	17	4	21	0.3968	0.50-0.75
3-15	21	5	26	0.4615	0.25-0.50
3-16	20	4	24	0.8889	0.25-0.50
3-19	19	5	24	0.2222	0.50-0.75
Totals	169	51	220	5.5733(10 df) 0.3879(1 df)	

* Adjusted X²-value

Family 1: homogeneity X² = 16.3422 with 7 df. P = 0.025-0.01

Family 2: homogeneity X² = 4.0523 with 8 df. P = 0.75-0.90

Family 3: homogeneity X² = 5.2144 with 9 df. P = 0.75-0.90

TABLE 4
 THE SEGREGATION RATIOS OF 26 R³LD F₂ LINES

Line	Number of Seedlings	
	Green	Albino
1- 2	53	3
1- 3	106	8
1- 6	92	7
1- 9	64	27
2- 1	64	13
2- 2	35	13
2- 3	24	17
2- 6	17	10
2- 7	34	21
2- 9	23	6
2-10	10	9
2-11	21	7
2-12	20	15
2-13	24	8
3- 2	37	17
3- 4	25	17
3- 5	18	10
3- 6	12	8
3- 9	29	24
3-10	54	30
3-11	59	23
3-12	37	9
3-13	13	1
3-14	41	19
3-15	36	15
3-16	47	10



frequency), and 3-16. The three segregating lines in the low frequency group gave an overall ratio of 251 green: 18 albino seedlings whereas the high frequency (except line 3-13) total was 148 green to 46 albinos, or frequencies of 6.7% and 23.8% albinos respectively.

A total of 43 plants from these seven lines were cloned with one clone of each plant being grown rapidly to maturity while the remaining clones were maintained at 38-42°F until the segregation could be determined so that only heterozygous plants were used as pollen parents. Of the 43 plants two failed to survive, of the remaining 41 plants 18 were found to be heterozygous as evidenced by segregation in the seedlings derived from the greenhouse grown clones.

The segregation occurring in the progeny of each of these 18 plants is shown in Table 5. The seven lines in the low frequency group gave an overall ratio of 447 green: 35 albino seedlings whereas the high frequency total was 215 green to 45 albinos, or frequencies of 7.3% and 17.3% albinos respectively.

The chromosome numbers of the green and albino seedlings in the segregating progeny were checked and all the albinos had 40 chromosomes whereas the green seedlings had from 40 to 42 chromosomes. On this basis the segregation ratios occurring in the lines shown in Tables 4 and

TABLE 5
 THE SEGREGATION RATIOS OF SEVEN CLONED R³LD F₃ LINES

R ³ LD F ₃ Line	Number of Seedlings	
	Green	Albino
1- 2-1	26	1
1- 2-3	37	2
1- 2-4	56	3
1- 2-6	40	1
1- 3-2	65	1
1- 3-3	77	2
1- 3-5	36	18
1- 3-8	60	1
1- 6-1	10	2
1- 6-4	39	3
1- 6-6	1	1
1- 9-1	24	5
1- 9-3	15	11
3-12-1	43	6
3-12-2	37	14
3-13-1	14	6
3-16-1	43	3
3-16-3	39	6

and 5 result from both genic segregation and chromosome number differences. This situation is very similar to that reported in Avena sativa by McGinnis and associates (8, 9 and 10). There are two possible explanations for these results; either the 40 chromosome albino plants were nullisomic and green 40 chromosome plants double monosomics or else all 40 chromosome plants were nullisomic and segregation occurred at a second locus. The fact that the frequency of albinos in these two groups was 6.7% and 23.8% respectively supports the latter theory. The high frequency monosomic group could be designated as aaB - wherein all 40 chromosome nullisomics would be aa-- and albino while the low frequency group could be designated AaB-, wherein $\frac{1}{4}$ of the nullisomics would be albino thus agreeing with the above frequencies.

III. THE F₁ HYBRIDS OF THE RESCUE MONOSOMICS X R³LD

All F₁ seedlings from the crosses between the Rescue monosomics and R³LD were checked for chromosome number. The data are summarized in Tables 6 and 7. From male parents with high albino frequency, the number of F₁ plants with 42, 41 and 40 (including 39) chromosomes was 112, 304 and 65 or 23.28%, 63.2% and 13.51% respectively. From male parents with low albino frequency, the number of F₁ plants with 42, 41 and 40 (including 39) chromosomes was 123, 324 and 51 or 24.7%, 65.06% and 10.24% respectively. The

TABLE 6

THE CHROMOSOME NUMBER AND MEIOTIC CONFIGURATIONS OF F₁ HYBRIDS OF
 RESCUE MONOSOMICS X R³LD LINES WITH HIGH ALBINO FREQUENCIES

F ₁ Line	Number of Seedlings	Chromosome Number and Meiotic Configuration				
		42	41	40		39
				19 ^{II} +2 ^I &/or 18 ^{II} +4 ^I	20 ^{II}	19 ^{II} +1 ^I &/or 18 ^{II} +3 ^I
R1A x R ³ LD	26	1	23	2		
R2A x R ³ LD	14	2	10	2		
R3A x R ³ LD	18	7	9	2		
R4A x R ³ LD	24	14	10			
R5A x R ³ LD	32	5	21	5	1	
R6A x R ³ LD	45	9	28	8		
R7A x R ³ LD	38	4	30	4		
R1B x R ³ LD	26	3	19	3	1	
R2B x R ³ LD	7		6	1		
R3B x R ³ LD	6	1	4	1		
R4B x R ³ LD	24	6	13	4	1	
R5B x R ³ LD	32	10	21	1		
R6B x R ³ LD	31	3	24	3	1	
R7B x R ³ LD	29	4	20	5		
R1D x R ³ LD	30	17	12	1		
R2D x R ³ LD	10	5	2	3		

continued

TABLE 6 CONTINUED

F ₁ Line	Number of Seedlings	Chromosome Number and Meiotic Configuration				
		42	41	40		39
				19 ^{II} +2 ^I &/or 18 ^{II} +4 ^I	20 ^{II}	19 ^{II} +1 ^I &/or 18 ^{II} +3 ^I
R3D x R ³ LD	9		8		1	
R4D x R ³ LD	10	4	2	4		
R5D x R ³ LD	39	7	26	6		
R6D x R ³ LD	19	5	11	3		
R7D x R ³ LD	12	5	5	2		
Totals	481	112	304	60	1	4