THIRD INTERNATIONAL WHEAT GENETICS SYMPOSIUM
SPONSORED BY THE AUSTRALIAN ACADEMY OF SCIENCE CANBERRA 5-9 AUGUST, 1968

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Proc. 3rd Int. Wheat Genet. Symp. Canberra 1968,
Aust. Acad. Sci. Canberra. pp. 299-304

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INTRODUCTION

Although speltoids and various other mutations are induced at high rates in hexaploid wheat by ionizing radiation, chlorophyll aberrations, which are among the most common mutations in diploids, are practically non-obtainable in wheat (MAC KEY, 1954). This is readily understandable if radiation-induced mutations are deficiencies, since all 21 nullisomics of wheat have substantially normal chlorophyll development (SEARS, 1954).

The only chlorophyll mutant of hexaploid wheat which has been intensively studied is Neatby's virescent (NEATBY, 1933), which occurred spontaneously in F_5 of an intervarietal hybrid and has been found to lie on the short arm of chromosome 3B (SEARS, 1956; STEINITZ-SEARS, 1963). It is a gene which actively interferes with chlorophyll formation, as shown by the fact that it allows normal chlorophyll production when present in one dose, leads to virescence in two doses, and causes extreme virescence or albinism in three doses (SEARS, 1956). Its normal allele has near duplicates on the homoeologous chromosomes, 3A and 3D (SEARS, 1957).

The discovery by J. G. HERMSEN of another virescent mutant in F_3 of a Timstein-5B Chinese Spring substitution line x Plantahof raises the question whether this may also involve chromosome 3B and whether it is likewise an allele that actively interferes with chlorophyll production. The authors gratefully acknowledge the kindness of Dr. HERMSEN, who supplied seed of this mutant and information concerning its origin.

Another mutant, called *chlorina* by its discoverers, SHAMA RAO and SEARS (1963), was obtained following treatment with ethyl methanesulfonate. Since other mutants phenotypically like *chlorina* are now in existence, it will be designated *chlorina*-1.

* Contribution from the Genetics Department, University of Missouri, and the Missouri Agricultural Experiment Station. Supported in part by National Science Foundation grant GB-3596. Journal Series No. 5428 of the Experiment Station.

CHLORINA-1

The preliminary data of SHAMA RAO and SEARS indicated that *chlorina*-1 was a simply inherited, mendelian recessive. It was crossed with the 21 different monosomics to determine which chromosome might carry it. Since there was reason to suspect that it might be a hemizygous-ineffective gene similar to Neatby's virescent—that is, one which would only be effective when present in two or more doses, particular attention was paid to the F_2 *chlorina* segregates. In the critical family these would all be disomic, whereas in all 20 other families they would be mostly monosomic and a few even nullisomic.

The first experiment failed to identify the chromosome involved. In every F_2 at least one *chlorina* segregate was found that was either monosomic or nullisomic (Table 1). In the 7A family, however, there were 10 *chlorinas* among 20 offspring, a departure from a 1 : 3 ratio approaching statistical significance.

TABLE 1. Data from F_2 populations from crosses of *chlorina*-1 with monosomics.

Chromosome concerned	Number grown	Number *chlorina*	Chromosome contribution of cytologically analyzed *chlorina* plants		
			21″	20″1′	other
1A	20	3		3	
1B	49	9		4	20″+t1″
1D	18	3		2	
2A	20	4	1	3	
2B	20	3	1	2	
2D	25	4		2	
3A	49	11		4	
3B	54	10		5	
3D	18	4		1	
4A	20	5		1	20″; 20″+t1″
4B	54	9	2	2	
4D	20	2			20″
5A	19	4	1	1	
5B	55	12		2	20″+t1″
5D	20	6	1	2	
6A	20	4	1	1	20″+i; 20″+t
6B	20	4		2	
6D	20	4			20″
7A	20	10*		3	20″+t
7B	20	3	1	1	20″
7D	15	1		1	
Totals (excl. 7A)	576	119			

*Presumably mostly hemizygotes.

A larger F_2 population from the F_1 involving mono-7A showed that the chlorophyll-defective plants fell into two classes, one of which was fully *chlorina* and the other more or less intermediate between *chlorina* and normal. When selected plants were examined cytologically (Table 2), it was clear that the gene is located on chromosome 7A. Of 17 full *chlorinas*, all but one

were disomic. The one exception, a monosomic, may be assumed to have been monosomic for some chromosome other than 7A, due to monosomic shift, or to have been incorrectly classified as *chlorina* rather than intermediate.

Further evidence that 7A is involved comes from the fact that only seven plants were fully green and that all seven of these were classified as nullisomic on the basis of their phenotype. In addition, two "gold", or extreme *chlorina,* plants were analysed, and each had an extra dose of one or both arms of a chromosome, presumably 7A. One was trisomic and the other had a disome composed of one normal and one isochromosome.

TABLE 2. Data from F_2 populations of crosses of *chlorina*-1 with monosomic 7A.

Number grown	Number green	Number light green	Number *chlorina*	Number gold
71	5	44	19	3
69	2	53	12	2

Chlorina-1 is clearly a gene which interferes with chlorophyll production, producing a light-green effect in one dose, *chlorina* in two doses, and gold in three. Like Neatby's virescent, it competes with its normal allele, as evidenced by the difference between hemizygote (light green) and heterozygote (green), but the difference is greater than between hemizygous and heterozygous virescent. Whether duplicates of the normal allele are present on the homoeologues, as with Neatby's virescent, has not yet been determined.

HERMSEN'S VIRESCENT

Hermsen's virescent is similar to Neatby's virescent but less extreme. Whereas Neatby's is lethal unless temperatures are fairly high, Hermsen's produces good plants in Missouri even during winter in the greenhouse.

Crosses with Chinese Spring monosomics showed that two recessive genes are involved and that these are located on chromosomes 3A and 3B. In F_2 the ratio of green to virescent (excluding the 3A and 3B crosses) was 15.1 : 1 (Table 3). For chromosome 3A, on the other hand, the ratio was 2.7 : 1, and for 3B, it was 2.6 : 1.

Data from four successive backcrosses to Chinese of Hermsen's virescent x Chinese Spring (Table 4) amply confirm that two recessive genes are involved. Following each backcross, one-fourth of the offspring were expected to be heterozygous for both genes and therefore able to segregate virescent offspring. Combining the data for the four generations, 7 of the 38 tested plants segregated. This is a reasonable fit to a 1 : 3 ratio; and an even better fit would presumably have been obtained had it been possible to test the remaining plants adequately.

The F_2 data from the crosses with mono-3A and -3B (Table 3) suggest that the two recessive genes for virescence behave as null alleles. If two doses of

each allele were required for the virescent phenotype (as is the case with Neatby's virescent), then the critical F_2's would segregate only about one-sixteenth virescents, for only the disomics could be virescent, and these would number only about 25%. On the other hand, if the genes concerned were null alleles, the F_2 disomics, monosomics, and nullisomics from the 3A and 3B crosses would all behave as homozygous recessives for the one gene, segregating only for the other. The cytological data showed, in fact, that virescent plants of all three constitutions did occur in the 3A and 3B F_2's.

TABLE 3. Data from F_2 populations of crosses of monosomics x Hermsen's virescent.

Chromosome concerned	Constitution of cytologically analyzed virescent segregates			No. of plants		Ratio
	21″	20″+1′	Other	Green	Virescent	
1A	1	2		67	3	1 : 22.3
1B		1	19″+il″+1′	66	2	1 : 33.0
1D		2		66	4	1 : 16.5
2A	1	1		67	2	1 : 33.5
2B		3		63	4	1 : 15.8
2D	1	2	19″+tl″+1′	31	4	1 : 7.8
3A	8	9	2 20″ 19″+2′ 20″+i′	78	29	1 : 2.7
3B	5	11	19″1‴ 20″+i	49	19	1 : 2.6
3D				116	7	1 : 16.6
4A	2	3		57	8	1 : 7.1
4B	1	3	19″+1‴+1′	65	5	1 : 13.0
4D		1		68	2	1 : 34.0
5A	4			59	4	1 : 14.8
5B	2	1		66	4	1 : 16.5
5D		3		65	5	1 : 13.0
6A	2	1	20″+t′	62	5	1 : 12.4
6B		3		61	3	1 : 21.0
6D		1	20″; 19″+2′; 19″+1‴+1′	81	4	1 : 20.3
7A	2	5		63	7	1 : 9.0
7B		1	20″ 15″+4‴+1⁗	66	3	1 : 22.0
7D		2	20″+il″	65	4	1 : 16.3

A cross of Hermsen's with Neatby's virescent was green in F_1 and segregated as follows in F_2: 21 green, 2 Hermsen (verified by backcrossing to parental lines), 6 apparently intermediate between Hermsen's and Neatby's, and 10 similar to Neatby's. Only one seed did not germinate. The rather large number of virescent segregates suggests, but does not prove, that there is an interaction between certain heterozygotes to produce the virescent phenotype. If, for example,

heterozygous Neatby's became virescent when homozygous for the Hermsen alleles on 3A, this would add one-half to the number of virescents and reduce the greens correspondingly.

Origin of Hermsen's Virescent

Since Chinese Spring, one of the parents of the hybrid from which Hermsen's virescent emerged in F_3, does not become virescent when it is made nulli-3A or -3B, it clearly is not mutant at either of the loci concerned. The other parent Plantahof, however, could well be homozygous for one of the mutant alleles.

TABLE 4. Data from backcrosses to Chinese Spring of Hermsen's virescent x Chinese Spring.

Backcross generation	Number plants tested	Number segregating	Segregation		Number plants in non-segregating families
			Green	Virescent	
1	8	2	2	38	15, 17, 19(2), 20(2)
2	11	2	2	40	5, 15, 19, 22, 26, 30(4)
3	10	2	3	67	34, 35(7)
4	9	1	2	37	26, 32, 36, 40(2), 41, 45, 46

Since no virescent segregates occurred among 42 F_2 offspring and only 10 of the 42 segregated in F_3 (data kindly supplied by J. G. HERMSEN), it is likely that the mutation at the second locus, which must have taken place in F_1, involved only part of the F_1 plant—in other words, it occurred at a later stage than in the zygote. As a result of segregation, two types of F_2 individuals occurred which were to segregate in F_3 or F_4: one type heterozygous for both loci and a second type homozygous for one mutant gene and heterozygous for the other. The former in turn gave rise to two types of segregating F_3 plants, and the latter only to F_3 individuals that segregated 3 : 1, if at all.

DISCUSSION

The simplest explanation for Hermsen's virescent is that it involves deficiencies (or null mutations) for the locus of Neatby's virescent on chromosome 3B and the locus of the near-duplicate of the normal allele of Neatby's on 3A. That this combination of deficiencies results in a virescent phenotype was demonstrated by SEARS (unpublished), who found that an X-ray-induced deficiency of v_1 on 3B was virescent when combined with nulli-3A. All the data thus far obtained are in accord with this explanation, but are not considered sufficient to establish the validity of the hypothesis.

It appears that the genes responsible for Hermsen's virescent cannot be used like Neatby's to reveal the occurrence of somatic loss and duplication of the chromosome concerned. Whereas Neatby's virescent is determined by a gene whose effect differs with dosages from one to three, the genes responsible for

Hermsen's virescent show no increase in effect with increasing dosage. The only type of plant in which somatic changes in dosage would be detectable would be one which was homozygous recessive for one Hermsen locus and heterozygous for the other. In such a plant, somatic loss of the chromosome carrying the one normal allele would result in a virescent sector.

Chlorina-1, however, behaves very similarly to Neatby's virescent in its manner of action and should be useful as an indicator of the somatic dosage of the chromosome concerned, 7A. Plants homozygous for *chlorina*-1 have a green or greenish stripe whenever one chromosome 7A is lost, and a gold stripe when a 7A becomes duplicated.

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