CYTOGENETICAL STUDIES IN WHEAT

VI.* CHROMOSOME LOCATION AND LINKAGE STUDIES INVOLVING *Sr13* AND *Sr8* FOR REACTION TO *PUCCINIA GRAMINIS* F. SP. *TRITICI*

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Abstract

Sr13 was located on the β arm of chromosome 6A and showed a recombination value of $0 \cdot 54 \pm 0 \cdot 07$ with the centromere. *Sr8* was localized to the opposite (α) arm and exhibited a recombination value of $0 \cdot 44 \pm 0 \cdot 05$ with the centromere. Genetic independence between *Sr13* and *Sr8* was confirmed in a genetic study involving a cross between two near-isogenic lines, each carrying one of the genes.

The use of rare chimaeric plants in monosomic populations for isolating marked chromosome misdivision products was demonstrated. The β telocentric arm of chromosome 6A, previously unavailable in any stock, was isolated by this means.

I. Introduction

Athwal and Watson (1956) found that common wheat (*Triticum aestivum* L.) cv. Khapstein possessed two genes, one dominant and one recessive, for resistance to certain cultures of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. isolated in Australia prior to 1954, and to a culture of Indian origin. North American studies (Knott 1962) indicated that Khapstein carried three genes for resistance, one of which was identified as *Sr7a*. The other two, previously unidentified, were designated *Sr13* and *Sr14*.

Following the development of near-isogenic lines carrying genes for resistance to *P. graminis* in the genetic background of cv. Marquis (Knott 1968) it has been established at this institution that the genes identified by Athwal and Watson were *Sr13* and *Sr14*. Of the three genes in Khapstein, only *Sr13* confers resistance to all Australian field cultures. Cultures collected since 1954 are virulent on *Sr14* and most are virulent on *Sr7a*.

This paper reports on the location, arm localization, and genetic relationship of *Sr13* and *Sr8*. The latter was previously located on chromosome 6A (Sears 1954; Sears, Loegering, and Rodenhiser 1957).

II. Materials and Methods

Khapstein W 1451 (W numbers refer to the Sydney University Wheat Accession Register) was crossed as the male parent to the Chinese Spring monosomic series. Although genes for resistance, which Khapstein inherited from its tetraploid parent, were expected to reside in the A or B genomes, the full series of crosses was made because earlier attempts to locate genes in Khapstein had proved unsuccessful.

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Cytological tests for validity of telocentric misdivision products obtained in F_2 and F_3 populations involving chromosome 6A were made in crosses with Chinese Spring plants ditelocentric for the α arm.

To test for allelism between *Sr13* and *Sr8*, the respective near-isogenic lines (W 2401 and W 2931) were crossed. Telocentric mapping of *Sr8* was performed by analysing the selfed progeny of a monotelodisomic (20″ + 1t″) plant from Chinese Spring monotelo-6A * 4/Mentana (pedigree system of Purdy *et al.* 1968) and the test cross progeny of a monotelodisomic plant from Chinese Spring monotelo-6A * 5/Mentana.

The *P. graminis* cultures utilized were chosen for appropriate pathogenic abilities. These were:

64726 (strain designation 116–4,5 on the system of Watson and Luig 1963, 1965).
68–L–4 (34–1,2,3,4,5,6,7)
70–L–5 (34–1,2,3,4,5,6,7)
334 (126–6,7)
70290 (21–5)
University of Missouri culture 59–51A (59–5,7).

All seven cultures are avirulent on seedlings with *Sr13* (infection type "2+3 ="). Virulence on seedlings with *Sr8* is denoted by "−6" in the strain designations for cultures 68–L–4, 70–L–5, and 334. Infection types produced when seedlings with *Sr8* were inoculated with avirulent cultures were "2" to "3 =".

Seedling populations were inoculated and tested by usual procedures. Mitotic studies were performed on root tips that had been treated in cold water or in a saturated solution of α-bromonaphthalene, fixed in Farmer's fixative, hydrolysed in 1N HCl, and stained in leuco-basic fuchsin. For meiotic studies, anthers were fixed in Farmer's fixative, hydrolysed in 1N HCl, and stained in leuco-basic fuchsin.

TABLE 1

SEGREGATION OF REACTION TO *PUCCINIA GRAMINIS* CULTURE 64726 IN F_2 POPULATIONS FROM MONOSOMIC F_1 PLANTS OF CROSSES BETWEEN CHINESE SPRING MONOSOMICS AND KHAPSTEIN

R, resistant; S, susceptible

Chromosome	Reaction		$\chi^2_{3:1}$*	Chromosome	Reaction		$\chi^2_{3:1}$*	Chromosome	Reaction		$\chi^2_{3:1}$*
	R	S			R	S			R	S	
1A	91	29	0·04	1B	71	36	4·26	1D	68	22	0·01
2A	87	25	0·43	2B	102	41	1·03	2D	68	22	0·01
3A	71	29	0·85	3B	64	21	0	3D	73	15	2·97
4A	78	38	3·72	4B	61	25	0·76	4D	52	37	13·04
5A	94	23	1·78	5B	88	28	0·04	5D	68	24	0·06
6A	98	18	5·56	6B	67	17	1·02	6D	66	20	0·14
7A	77	37	3·38	7B	74	19	1·04	7D	61	28	1·98

Total (excluding 6A): 1481 resistant, 536 susceptible, $\chi^2_{3:1}$ 2·66

* Values for significance: 3·84 ($P = 0·05$); 6·64 ($P = 0·01$).

III. RESULTS

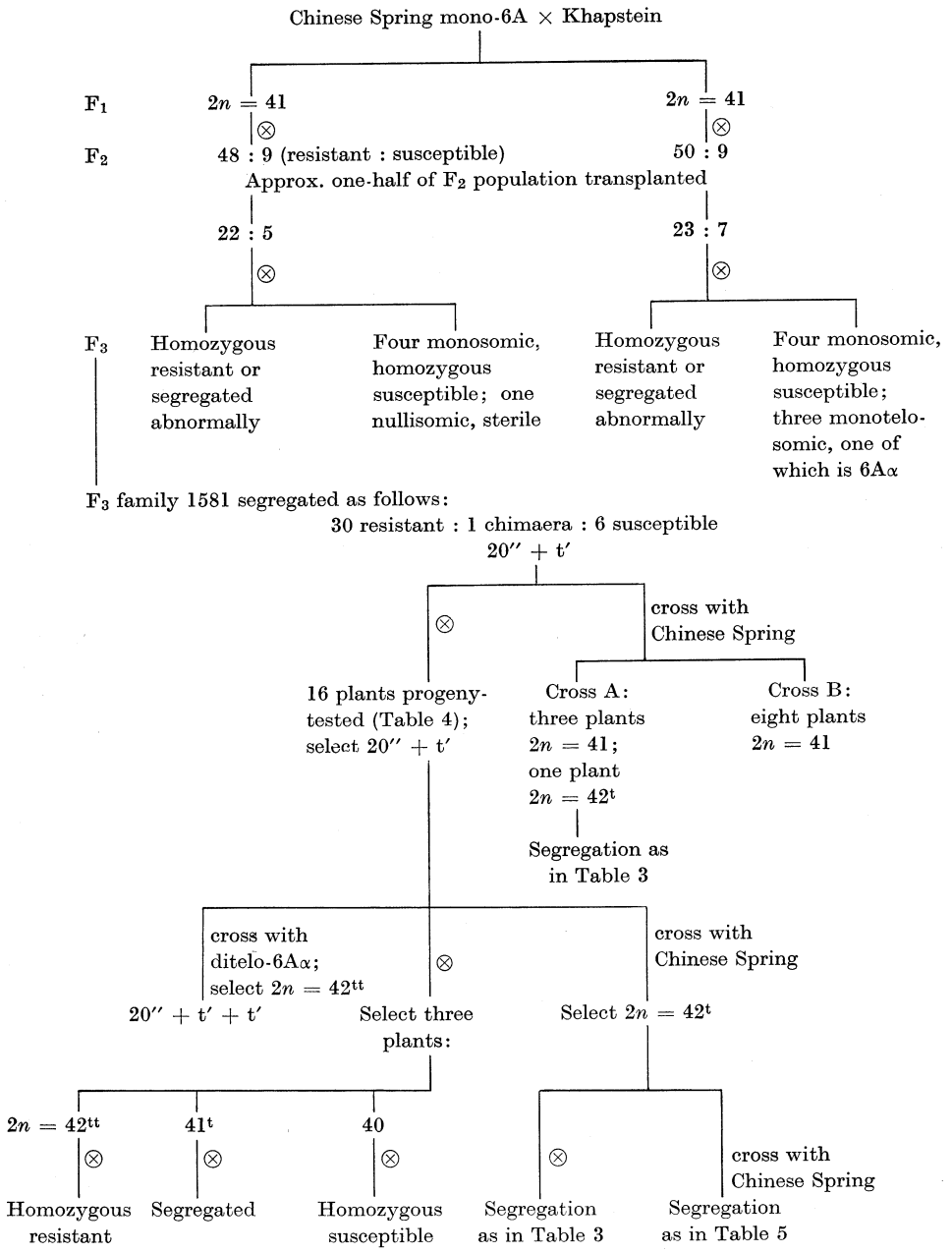
(a) *Chromosome Location of* Sr13

Seedling segregation ratios in progenies of F_1 plants from crosses between the various Chinese Spring monosomics and Khapstein (Table 1) deviated from those expected at the $P = 0·05$ level for single-factor pair segregation in three instances.

TABLE 2

SUMMARY OF STEPS INVOLVED IN ANALYSIS OF *Sr13*

⊗ = selfed cross



Ratios for chromosomes 1B and 4D deviated in the direction of excess susceptible seedlings, whereas for the critical cross, a deviation in the opposite direction was expected. Hence chromosome 6A appeared to be involved, but as this result was not considered to be conclusive, approximately one-half of the F_2 populations were transplanted to obtain F_3 lines for further study.

F_3 data, obtained using culture 68–L–4, confirmed that *Sr13* was located in chromosome 6A. Mitotic chromosome counts on two, three, or four seedlings within each F_3 line from the 6A cross permitted deduction of the chromosome constitution of each F_2 plant. Disomic F_2 plants which were resistant produced homozygous resistant F_3 families, whereas monosomic F_2 plants which were resistant gave progenies with clearly abnormal segregation ratios. On the other hand tests on F_3 lines from the 20 non-critical crosses confirmed single-gene segregation in each instance.

The F_2 data for chromosome 6A and the various steps involved in subsequent studies are summarized in Table 2. Of the 18 susceptible seedlings, in the F_2 population involving chromosome 6A, 12 were transplanted. One of these was nullisomic in appearance and proved to be sterile. From mitotic studies of progenies it was inferred that eight were monosomic, while three, which had been nullisomic-like but partially fertile, were inferred to have been monotelosomic. The telocentric derivative in one of these was identified as 6Aα. Since these derivatives were susceptible to *P. graminis*, the test established that the population was indeed aneuploid for chromosome 6A and that *Sr13* was not located in the α arm. The inclusion of eight presumed monosomic plants in the susceptible group was unexpected, but since their constitutions were determined from mitotic chromosome counts on progenies, other explanations are possible. These plants may have been monoisosomic—an isochromosome would not be identified somatically—or they may have been nullisomic for chromosome 6A and trisomic for a compensatory homoeologous chromosome. However, their normal plant vigour reduced the first possibility. A more likely explanation is that they resulted from outcrossing—the pollination of 20-chromosome eggs lacking chromosome 6A by 21-chromosome pollen grains carrying the susceptible allele from a plant in another cross or from an outside source. Poor fertility of many of the monosomic F_1 plants from the cv. Chinese Spring $\times$ Khapstein crosses was noted and definite instances of outcrossing were established in certain crosses.

(*b*) *Telocentric Mapping of* Sr13

A seedling displaying a chimaeric reaction to *P. graminis* appeared in one F_3 family from a resistant F_2 plant. This seedling was transplanted and was found to have 20 bivalents and a telocentric univalent chromosome (20″ + t′) at meiosis. Two spikes were pollinated with cv. Chinese Spring and the remaining spikes were permitted to self.

The telocentric chromosome was recovered in only one (1581/Chinese Spring A.4) of 12 seedlings obtained from the crosses. Chromosome counts and seedling reactions of 19 progeny of this individual are presented in Table 3. Mitotic chromosome counts were obtained for 16 selfed seedlings from the chimaera. Their frequencies, meiotic constitutions, and behaviour when progenies were tested, are given in Table 4. Because of trisomy of the ditelocentric individual, plants with 20″ + t′ were chosen for further study. Firstly an individual with two telocentric

chromosomes from a cross with a Chinese Spring plant ditelocentric for 6Aα displayed, at meiosis, 20 bivalents and two telocentric univalents (20″ + t′ + t′). This established that the telocentric being tested involved chromosome 6A and that it was the β

TABLE 3

CHROMOSOME CONSTITUTIONS AND REACTIONS TO *P. GRAMINIS* CULTURE 70–L–5 IN PROGENIES OF MONOTELODISOMIC PLANTS HETEROZYGOUS FOR *Sr13*

Chromosome Constitution	Reactions of 1581/Chinese Spring A.4 progeny		Reactions of 1581.7/Chinese Spring progeny	
	Resistant	Susceptible	Resistant	Susceptible
42^{tt}*	1	—	1	—
42^{t}†	9	1	4	2
42	5	1	11	4
41	1	1	1	—
Total	16	3	17	6

* Including two telocentric chromosomes.
† Including one telocentric chromosome.

arm of this chromosome. Secondly, three plants were selected from a selfed monotelocentric individual. One was ditelocentric and progeny tests established that it was homozygous for *Sr13*. One was monotelocentric and mitotic counts of nine progeny showed four with $2n = 41^{t}$ and five with $2n = 40$. The 41^{t} seedlings were

TABLE 4

CHROMOSOME CONSTITUTIONS, MEIOTIC CONFIGURATIONS, AND REACTIONS TO *P. GRAMINIS* CULTURE 70–L–5 OF 16 PLANTS FROM THE SELFING OF *Sr13* CHIMAERA

No. of plants	Mitotic count*	Meiotic configuration*	Progeny test
1	$2n = 43^{tt}$	19″ + 1‴ + t″	Homozygous resistant
1	$2n = 42^{t}$	20″ + i′ + t′ or 20″ + it″	Segregating
1	$2n = 41$	20″ + i′	Segregating
10	$2n = 41^{t}$	20″ + t′	Segregating
3	$2n = 40$	20″	Homozygous susceptible

* t = telocentric; i = isochromosome; ′ = univalent; ″ = bivalent; ‴ = trivalent.

resistant, and those with 40 chromosomes susceptible, to culture 70–L–5. Three seedlings with $2n = 40$ from a third plant with 20″ were susceptible as expected. This study conclusively demonstrated that *Sr13* was located in the telocentric chromosome.

In a third study involving a cross with Chinese Spring, a monotelodisomic progeny was further test-crossed with Chinese Spring. Table 5 lists the somatic counts and reaction frequencies of the progeny. One susceptible individual with $2n = 43^t$, was considered to be a recombination. Of two seedlings with $2n = 41^t$, one was considered a recombinant and the other a parental type. As the telocentric chromosome was transmitted, aneuploidy must have involved a different chromosome. Of two plants with $2n = 41$, the resistant individual was considered a recombinant, whereas the second, being susceptible, could not be included in the analysis since it may have been deficient, rather than parental, for chromosome 6A. Hence of 20 gametes sampled, 12 were recombinant, indicating that *Sr13* is independent of the centromere (recombination = $0{\cdot}60 \pm 0{\cdot}11$).

TABLE 5

CHROMOSOME CONSTITUTIONS AND REACTIONS TO *P. GRAMINIS* CULTURE 70–L–5 OF 21 SEEDLINGS FROM TEST-CROSS OF HETEROZYGOUS MONOTELODISOMIC PLANT

Chromosome constitution	Reaction*	
	Resistant	Susceptible
43^t	—	1 R
42^t	4 P	6 R
41^t	1 P	1 R
42	3 R	3 P
41	1 R	1 —

* R = recombinant; P = parental.

Data for 23 selfed progeny from the test-crossed plant (1581.7/Chinese Spring) are included in Table 3. Recombination based on the method of maximum likelihood for the combined data in Table 3, but omitting individuals with $2n = 41$, was estimated to be $0{\cdot}49 \pm 0{\cdot}10$. A recombination estimate using the pooled test-cross and self data was $0{\cdot}54 \pm 0{\cdot}07$.

(c) *Linkage of* Sr8 *and* Sr13

Thirty-six F_3 lines from a cross between the appropriate near-isogenic lines were tested with culture 334 which is virulent on plants with *Sr8*, and with culture 64726 which is avirulent on plants with either *Sr8* or *Sr13*. Because of low seedling numbers in some lines, determinations as to whether lines were homozygous resistant or segregating were not possible, especially with the second culture where two-gene segregation was expected. Hence lines were classified into three groups, the expected frequencies for which, if independence is assumed, are:

12 homozygous resistant or segregating with both cultures, i.e. genotypes *Sr13Sr13* — — and *Sr13sr13* — —;

3 homozygous resistant or segregating with culture 64726 only, i.e. genotypes *sr13sr13 Sr8Sr8* and *sr13sr13 Sr8sr8*;

1 homozygous susceptible with both cultures, i.e. genotypes *sr13sr13 sr8sr8*.

The realized ratio of 30 : 4 : 2 does not differ significantly from the expected distribution ($\chi^2_{3:1}$ (30 : 6) = 1·33; $P > 0·25$). This result indicated that *Sr13* and *Sr8* are not linked.

(*d*) *Telocentric Mapping of* Sr8

A monotelodisomic plant from the cross Chinese Spring monotelo-6Aα * 5/Mentana (a $2n = 41$ individual being selected for each backcross) was test-crossed with Chinese Spring. Mitotic chromosome counts and reactions of the progenies with culture 70290, which is avirulent on seedlings with *Sr8*, are given in Table 6. Among 91 gametes sampled, 41 recombinants were recovered. Recombination between *Sr8* and the centromere was estimated to be 0·46±0·05.

TABLE 6

MITOTIC CHROMOSOME COUNTS AND REACTIONS TO *P. GRAMINIS* CULTURE 70290 OF TEST-CROSS PROGENIES OF MONOTELODISOMIC PLANT FROM CHINESE SPRING MONOTELO-6Aα * 5/MENTANA

Direction of cross	Chromosome No.	Reaction†	
		Resistant	Susceptible
Male	42	16	10
	42t	—	3
Female	42	14	12
	42t	20	16
Total	42	30 P	22 R
	42t	20 R	19 P

† P = parental; R = recombinant.

TABLE 7

CHROMOSOME COUNTS AND REACTIONS TO *P. GRAMINIS* CULTURE 59–51A OF PROGENY OF SELFED MONOTELODISOMIC PLANT FROM CHINESE SPRING MONOTELO-6Aα * 4/MENTANA

Chromosome No.	Reaction		Total
	Resistant	Susceptible	
42	22	4	26
42t	13	2	15
42tt	1	1	2
Total	36	7	43

A selfed population from a monotelodisomic plant in cross Chinese Spring monotelo-6Aα * 4/Mentana was scored mitotically for chromosome number and tested with culture 59–51A. Frequencies and reaction classes are presented in Table 7. Some reactions considered doubtful on a single-plant basis were confirmed by

progeny testing. Recombination, based on the method of maximum likelihood, was estimated at $0{\cdot}37 \pm 0{\cdot}09$.

Using the pooled test-cross and self data, recombination between *Sr8* and the centromere was estimated to be $0{\cdot}44 \pm 0{\cdot}05$.

IV. Discussion

Genes *Sr8* and *Sr13* concerned with reaction to *P. graminis* were located in opposite arms of chromosome 6A. *Sr8* was localized to the α (standard) arm and showed recombination of $0{\cdot}44 \pm 0{\cdot}05$ with the centromere, whereas the estimate of $0{\cdot}54 \pm 0{\cdot}07$ suggests that *Sr13* is independent of the centromere in the β arm. These findings were supported by a concurrent genetic study indicating that *Sr8* and *Sr13* were independently inherited.

The studies with *Sr13* demonstrated the value of occasional chimaeric plants which may appear in segregating populations. Such chimaeras frequently carry chromosome misdivision products which can be used for chromosome arm determinations and, if telocentric as in this study, for telocentric mapping. However, misdivision products are not always stable. From a chimaeric seedling with part of its tissue having a telocentric, or isochromosome, bearing the particular dominant (or hemizygous effective) marker, subsequent growth appears to be random. Hence the misdivision products are not always recovered, or they may be somatically unstable (Steinitz-Sears 1966). The detection of a chimaera in these studies not only permitted the determination of the particular arm bearing *Sr13*, but also allowed the isolation of a telocentric chromosome which was previously unavailable in wheat. Moreover, the newly isolated telochromosome is marked with *Sr13* which should enhance its value for future mapping purposes. As the result of recombination and further selection, ditelocentric 6Aα stocks homozygous for *Sr8* also should be available.

Although *Sr13* confers resistance to all current Australian field strains of *P. graminis* there has been difficulty in exploiting it as a resistance source in commercial wheat cultivars. A physiological "black-chaff" condition appears to be associated with its presence. This detracts from agronomic appearance and, under certain conditions at least, undoubtedly leads to yield depression. In these studies, the black-chaff condition has persisted in *Sr13*—bearing monotelodisomic individuals after backcrossing to Chinese Spring. This association requires further investigation to determine the intensity of linkage, and to determine the relationship, if any, between the black-chaff characteristic and leaf necrosis characters which have been associated with chromosomes of homoeologous group 6 (Sears 1954, 1966; Morris, Schmidt, and Johnson 1970; Wenzel 1971). A well-known black-chaff phenotype allegedly linked with field resistance to *P. graminis* has been associated with chromosome 3B of cv. Hope which, like Khapstein, resulted from a cross of tetraploid with hexaploid wheat, but there is no evidence to suggest these occurrences are related in any way.

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