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Cytogenetical Studies in Wheat. IX.* Monosomic Analyses, Telocentric Mapping and Linkage Relationships of Genes *Sr21*, *Pm4* and *Mle*

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Abstract

Gene *Sr21* for resistance to *Puccinia graminis tritici* in five hexaploid derivatives of the diploid *Triticum monococcum* was located in chromosome 2A. Since only one chromosome was involved in resistance, abnormal ratios found in some diploid wheat crosses and in one hexaploid derivative were attributed to differential transmission of gametes rather than to gene duplication in the diploid wheat sources. By using 2AS and an unknown telocentric presumed to be 2AL in telocentric mapping, *Sr21* was placed $2{\cdot}4 \pm 0{\cdot}9$ recombination units from the centromere in 2AL.

Genes *Pm4* and *Mle* for resistance to *Erysiphe graminis tritici* were also located in chromosome 2A. Attempted telocentric mapping of *Pm4* with the 2AS telosome suggested that *Pm4* was situated either very close to the centromere in 2AS, or somewhere in 2AL. The latter possibility was confirmed when *Pm4* showed $37{\cdot}5 \pm 1{\cdot}7\%$ recombination with *Sr21*. *Mle* was located in chromosome 2AL and segregated independently of the centromere. *Pm4* and *Mle* behaved as alleles. Consequently, *Pm4* was redesignated *Pm4a* and *Mle* was designated as *Pm4b*.
[Other keywords: leaf rust, powdery mildew, stem rust.]

Introduction

Cultivar Einkorn of diploid wheat, *Triticum monococcum* L., is one of 12 wheat genotypes chosen by Stakman *et al.* (1962) to distinguish physiological races of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., the wheat stem rust pathogen. The gene *Sr21* for resistance to *P. graminis tritici* in diploid wheat was transferred to hexaploid bread wheat, *T. aestivum* L., by The (1973) who also reported its location in chromosome 2A, one of the seven chromosomes of the A genome which this species shares with *T. monococcum*. Bread wheat seedlings with *Sr21* distinguish physiological races of *P. graminis tritici* in an identical manner to Einkorn, but the disease phenotypes and the low infection types are less resistant than those produced by the diploid.

Briggle (1966) reported the transfer of a gene designated *Pm4* for resistance to *Erysiphe graminis* D.C. f. sp. *tritici* emend. Marchal, from the tetraploid *T. turgidum* L. wheats Khapli emmer and Yuma durum to the hexaploid winter wheat cultivar Chancellor. Wolfe (1967) lists three bread wheat genotypes with a mildew resistance gene designated *Mle*, namely Weihenstephan M1, ELS and TP229. At the Plant Breeding Institute, Castle Hill, N.S.W., work has been in progress to transfer *Pm4* and *Mle* to the Australian spring wheat Federation by backcrossing. In addition, a gene subsequently shown to be identical with *Mle* on the basis of tests with the

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pathogen was transferred to Federation from the Sydney University accession W804 of Persian wheat (*T. turgidum*).

This paper presents results for the chromosome location of *Sr21*, *Pm4* and *Mle* in chromosome 2A and reports their positions relative to the 2A centromere and with each other.

Materials and Methods

The following stocks were used in crosses with monosomics, monotelosomics or monoisosomics (W numbers refer to the Sydney University Wheat Accession Register):

Sr21: Five hexaploid wheat stocks with resistance to *P. graminis tritici* derived from different accessions of *T. monococcum*, namely:

W3586, *T. turgidum* L. W304/*T. monococcum* W10/2/unknown *T. aestivum*.

W3588, *T. turgidum* cv. Spelmar*2/*T. monococcum* G21/2/2**T. aestivum* cv. Steinwedel W199.

W3591, *T. aestivum* W1569/*T. monococcum* C68.123/2/*T. aestivum* cv. Chinese Spring W1806.

W3694, Steinwedel/2/W1569*2/*T. monococcum* C68.124.

W3695, W1569*3/*T. monococcum* C68.125.

On the basis of the infection types produced with several strains of *P. graminis tritici* all five stocks appeared to carry *Sr21*. However, genetic studies on some of the diploid donors suggested that duplicate genes might be involved. W3591 was used as a standard in later studies.

Pm4: Khapli/8*Chancellor, W3145.

Mle: A homozygous mildew resistant F_3 line in Federation*7/W804

Since *Sr21* was assumed to be located in one of the A-genome chromosomes crosses were made only with A-genome aneuploids, and since *Pm4* and *Mle* were each assumed to be located in a chromosome of the A or B genomes crosses were made with A- and B-genome aneuploids. *T. aestivum* shares the A genome with *T. monococcum* and *T. turgidum* and the B genome with *T. turgidum*. The majority of Chinese Spring aneuploids, monosomics (CSM), monotelosomics (CSMT), monoisosomics (CSMI) and ditelosomics (CSDT) were originally supplied by Dr E. R. Sears, University of Missouri, U.S.A., but one monotelosomic (CSMT2AT) arose spontaneously in CSM2A and one plant monotelodisomic for chromosome 2AL, having 41 normal chromosomes plus the longer telosome for 2A, had been produced from an earlier hybrid involving a cross with a 43-chromosome plant ditelosomic 2AL and monotelosomic 2AS. In Chinese Spring wheat the CSDT2AL condition is not viable and is therefore maintained in the presence of monotelo-2AS.

Steinwedel, Chinese Spring and Federation were used as susceptible parents in genetic studies. For tests of linkage and allelism various homozygous and heterozygous stocks were intercrossed, depending on the availability of appropriate plants.

Somatic chromosome counts were determined on excised root tips, pretreated in ice water and prepared by the Feulgen procedure. Meiotic examinations were performed on pollen mother cell smears.

Individual F_1, F_2, or testcross seedlings and F_3 populations were inoculated with *P. graminis tritici* or *E. graminis tritici* at the first seedling leaf stage. Where results with both pathogens were desired, inoculation with *P. graminis tritici* preceded that with *E. graminis tritici* by 6–10 days. The main *P. graminis tritici* culture used was 66-L8, a yellow uredospore variant of strain 34-ANZ-2,4,5,6 which is avirulent on seedlings of Einkorn and the five *T. monococcum* derivatives listed above [for strain designation in Australia see Watson and Luig (1963) and Luig and Watson (1977)]. For Australian mildew studies *E. graminis tritici* culture S.U.1 (McIntosh and Baker 1966) was used. This culture is avirulent on seedlings with *Pm4* or *Mle*. At Cambridge three cultures were used, namely W76/95, avirulent for *Pm4* and *Mle*; N76/139, avirulent for *Mle* and virulent for *Pm4*; and W76/163 which is virulent for both host genes. Studies with W76/95 were performed by infecting entire seedlings in pots in a greenhouse whereas studies with the other two Cambridge cultures involved inoculations of small leaf segments maintained on benzimidazole-impregnated agar developed from methods described by Wolfe (1967).

Results

Monosomic Analyses

(i) *Sr21*

The results of monosomic analyses for the five *T. monococcum* derivatives are presented in Table 1. The F_2 segregation ratios for the six monosomic crosses, excluding 2A, for each stock were homogeneous. Consequently only the pooled data are presented. In the case of W3588, W3591, W3694 and W3695 the results clearly showed that the F_2 segregation ratios involving monosomic 2A were significantly different from the crosses involving Chinese Spring or the other monosomics, suggesting the location of *Sr21* in chromosome 2A in each of the genotypes tested. In the case of W3586 the results for the crosses with Chinese Spring and with the six

Table 1. F_2 segregation for seedling reaction to *P. graminis tritici* culture 66-L-8 in crosses involving five *T. monococcum* derivatives with Chinese Spring and the A-genome Chinese Spring monosomics or monotelosomics

Cultivar tested		Chinese Spring crosses	Monosomic F_1's excl. 2A crosses	Monosomic F_1's 2A cross only
W3588	Resistant	123	704	103
	Susceptible	38	211	2
	$\chi^2_{3:1}$	0·17	1·84	29·87**
W3591	Resistant	959	989	226
	Susceptible	311	289	16
	$\chi^2_{3:1}$	0·17	3·87	43·64**
W3694	Resistant	295		309
	Susceptible	99		9
	$\chi^2_{3:1}$	0·00		83·36**
W3695	Resistant	120		201
	Susceptible	37		15
	$\chi^2_{3:1}$	0·17		37·56**
W3586	Resistant	312	1143	228
	Susceptible	68	271	15
	$\chi^2_{3:1}$	10·09**	26·05**	45·96**

** χ^2 value for significance at $P = 0{\cdot}01$ is 6·63.

monosomics, excluding 2A, deviated significantly from expectation for single-gene segregation. Nevertheless, comparisons of the three sets of data by means of contingency table χ^2 tests (Mather 1951) indicated that segregation in monosomic 2A was significantly different from that in the euploid cross ($\chi^2 = 17{\cdot}6$, $P < 0{\cdot}001$), and from that for the pooled ratio from the six monosomics other than 2A ($\chi^2 = 24{\cdot}5$, $P < 0{\cdot}001$).

(ii) *Pm4*

None of the F_2 segregations for the 14 monosomic populations showed a statistical deviation from a 3 resistant [infection type (I.T.) '0'] : 1 susceptible (I.T. '3' or '4') ratio of the type which would have identified the critical chromosome. F_1 monosomics from residue seed of 12 of the original crosses were pollinated with the

corresponding ditelocentric lines of Chinese Spring. These tests enabled correlations of mildew reaction and chromosome number (Table 2) and if necessary would have also enabled a check of the validity of the particular monosomic F_1's. In the chromosome 2A cross all plants with $2n = 41+t$ were resistant whereas sib plants with $2n = 40+t$ were susceptible. Subsequent growth characteristics indicated that these latter plants were monotelosomic for chromosome 2A. The failure to obtain a disturbed F_2 ratio from the monosomic 2A cross apparently resulted from a high nullisomic 2A frequency and failure to recognize the susceptible seedlings as nullisomics at early growth stages.

Table 2. Mitotic chromosome counts and reactions to *E. graminis tritici* culture S.U.1 in testcrosses of various Chinese Spring monosomic/2/ Khapli/8*Chancellor monosomic hybrids with corresponding ditelocentric lines

R, resistant; S, susceptible; n.t., not tested

Chromosome designation	Chromosome count $2n = 41+t$		Chromosome count $2n = 40+t$	
	R	S	R	S
1A	—	—	3	1
2A	9[A]	—	—	12
3A	—	1	1	3
4A	n.t.			
5A	—	—	1	3
6A	1	1	1	3
7A	1[A]	2	1	—
1B	1	1	1	
2B	1	—	13	8
3B	1	1	1	2
4B	—	1	—	4
5B	n.t.			
6B	2	—	1	2
7B	1	—	1	2
Total excl. 2A	8	7	24	28

[A] Includes one plant with $2n = 42+t$ (meiosis $19''+1''+1t''$).

(iii) *Mle*

Again, F_2 segregations in the progenies of monosomic F_1's failed to deviate from 3 : 1 ratios. F_2 segregations in progenies of three monosomic plants in the 2A cross were 5 resistant (I.T. '01−') : 0 susceptible, 23 : 2 and 19 : 7 whereas the progenies of two euploid sister plants segregated 22 : 6 and 19 : 11. Six of the nine susceptible plants from the monosomic parents appeared nullisomic. The third monosomic plant above had been pollinated with Chinese Spring. Twelve seedlings with $2n = 42$ were mildew resistant whereas 15 seedlings with $2n = 41$ and one with $2n = 41+t$ were susceptible. Since only those seedlings obtaining all 21 entire chromosomes from the monosomic parent were resistant whereas those obtaining $n = 20$ or $20+t$ were susceptible it was concluded that *Mle* was present in chromosome 2A. Presumably, the telocentric chromosome resulted from misdivision of the 2A monosome and represented the chromosome arm not carrying *Mle*.

Telocentric Mapping

(i) *Sr21*

The spontaneously occurring monotelosomic plant CSMT2AT was not examined meiotically but mitotic chromosome counts on seedlings derived from the few selfed seeds showed two with $2n = 40+t$, one with $2n = 39+t$ and one with $2n = 38+t$. Of 15 seedlings from the cross with W3591, six had $2n = 41+t$ and nine had chromosome numbers ranging from 38 to 44, with five possessing the telocentric. Hence the CSMT2AT must have been meiotically irregular, further indicating the possible absence of the short arm of chromosome 2A which bears a gene for normal synapsis (Sears 1954), and suggesting that the monotelosome 2AT was the long arm, namely 2AL. Visual comparisons of this telocentric with the standard 2AS telocentric in different plants revealed its greater length.

Table 3. Chromosome constitutions and reactions of seedlings to *P. graminis tritici* culture 66-L-8 in crosses enabling the telocentric mapping of *Sr21*

Analysis A: CSMT2AT/W3591, monotelodisomic ($2n = 41+t$)/2/W199. Analysis B: W199/2/CSMT2AT/W3591, monotelodisomic. Analysis C: F_2 of resistant monotelodisomic recombinant in A. Analysis D: F_2 of CSDT2AS/W3591. R, resistant; S, susceptible

Chromosome constitution[A]	Analysis A		Analysis B		Analysis C		Analysis D	
	R	S	R	S	R	S	R	S
42	69	2	92	1	4	32	103	—
41+t	1	49	0	7	32	0	94	—
40+2t	—	—	—	—	1	0	—	2
Sub-total	70	51	92	8	37	32	197	2
43	1	—	1	—	—	—	—	—
42+t	—	—	3	—	2	—	5	—
41+2t	—	—	—	—	—	—	1	—
40+t+t	—	—	—	—	—	—	1	—
41	1	6	5	2	—	2	3	—
40+t	—	2	—	—	3	—	—	—
Total	72	59	101	10	42	34	207	2

[A] 42, 41+t, 40+2t, etc. indicate the number of normal chromosomes and telocentrics; 2t represents indistinguishable telocentrics whereas t+t indicates two distinguishable telocentrics.

In monotelodisomic plants telocentric chromosomes are usually transmitted normally through the eggs but in pollen they are at a disadvantage when competing with normal counterparts. Hence the proportion of progeny carrying the telocentric varies with the direction of testcrossing and with the actual telocentric involved. Testcross results for one monotelodisomic F_1 plant from CSMT2AT/W3591 used as female and as male parent appear in Table 3, analyses A and B respectively. In this cross the *Sr21* allele was carried by the normal 2A homologue. Of three apparent recombinants observed in analysis A, one with $2n = 41+t$ was resistant. Considering only those plants with 42 chromosomes this represents a recombination value of $3/121 = 2{\cdot}5 \pm 1{\cdot}4\%$ between *Sr21* and the centromere. When the monotelodisomic F_1 was used as the male parent the telosome was transmitted in only 7% of the 21-chromosome gametes. The recombination value between *Sr21* and the centromere was $1{\cdot}0 \pm 1{\cdot}0\%$.

Table 3 analysis C lists the chromosome numbers and reaction class frequencies for F_2 seedlings when the recombinant resistant monotelodisomic plant in analysis A was selfed. In this case *Sr21* should be present in the telocentric chromosome. Among 69 plants with 42 chromosomes all plants with one or more telocentrics and four of 36 plants with only normal chromosomes were resistant. Using the method of linkage estimation described by The and McIntosh (1975) recombination between *Sr21* and the centromere was determined to be $4{\cdot}0 \pm 2{\cdot}0\%$.

A pooled estimate of linkage of $2{\cdot}4 \pm 0{\cdot}9\%$ was determined from the results of analyses A, B and C.

Table 3 analysis D gives results for the CSMT2AS cross. Among 199 F_2 seedlings with 42 chromosomes no recombination products were found, the result expected if *Sr21* is located in chromosome 2AL.

Table 4. Chromosome constitutions and reactions of seedlings to *E. graminis tritici* culture S.U.1 in testcrossed and selfed populations of plants heterozygous for *Mle* and monotelodisomic for chromosome 2AL

Chromosome constitution	Reaction	Test-crossed as male parent	Test-crossed as female parent	Total	Selfed	
42	Resistant	8	24	32	35	41
	Susceptible	9	19	28[A]	6[A]	
41+t	Resistant	1	21	22[A]	34	44
	Susceptible	3	18	21	10[A]	
40+2t	Resistant	—	—	—	6[A]	6
	Susceptible	—	—	—	—	
Total		21	82	103	91	
41+2t	Resistant				1	
42+t	Resistant				1	
41	Resistant				3	
	Susceptible		3		3	
40+t	Resistant				1	

[A] Recombinant phenotypes.

In each analysis there were several seedlings possessing chromosome numbers other than 42. These presumably resulted from meiotic instability and, as their origins were not ascertained, they were omitted from linkage estimations. Further observations were made on some of these individuals and the results did not conflict with the conclusions reached.

(ii) *Pm4*

Eighty-one F_2 seedlings derived from three mildew-resistant monotelodisomic individuals from the CS monosomic-2A/2/Khapli/8*Chancellor, $2n = 41/3/CS$ ditelosomic 2AS testcross were scored for chromosome number and mildew reaction:

$2n = 42$	41 seedlings	Resistant
$2n = 41+t$	34 seedlings	Resistant
	1 seedling	Susceptible
$2n = 40+2t$	5 seedlings	Susceptible

These results indicated that *Pm4* must be located near the centromere in chromosome 2AS, or on the opposite arm, but another method of locating *Pm4* was considered necessary. Although the one susceptible plant with $2n = 41+t$ could have resulted from genetic recombination the presence of a single individual of this type may have resulted from technical error, or from 'biological error' involving a more complex level of aneuploidy.

(iii) *Mle*

A mildew-susceptible plant known to be monotelodisomic for chromosome 2AL was pollinated with Federation*7/W804. Three mildew-resistant hybrids with $2n = 41+t$ and 20 normal bivalents and a heteromorphic bivalent in most pollen mother cells at meiotic metaphase were testcrossed with Federation and selfed. Mitotic chromosome numbers and mildew reactions are summarized in Table 4. Clearly, the within-chromosome group genetic ratios conformed closely with 1 : 1 for the testcross and with 3 : 1 for the selfed populations indicating that segregation for *Mle* was independent of the 2A centromere.

Table 5. Observed genotypic frequencies and their expected proportions assuming recombination value *r* separating genes *Sr21* and *Pm4* in cross Federation*4/2/Yuma/8*Chancellor/3/W3591 when tested with *P. graminis tritici* culture 66-L-8 and *E. graminis tritici* culture S.U.1

Testcross populations			F_3 populations		
Genotype	Expected	Observed	F_2 genotype	Expected	Observed
Sr21 sr21 Pm4 pm4	$\frac{1}{2}r$	78	*Sr21 Sr21 Pm4 Pm4*	$\frac{1}{4}r^2$	13
Sr21 sr21 pm4 pm4	$\frac{1}{2}(1-r)$	119	*Pm4 pm4*	$\frac{1}{2}r(1-r)$	53
sr21 sr21 Pm4 pm4	$\frac{1}{2}(1-r)$	104	*pm4 pm4*	$\frac{1}{4}(1-r)^2$	37
sr21 sr21 pm4 pm4	$\frac{1}{2}r$	63	*Sr21 sr21 Pm4 Pm4*	$\frac{1}{2}r(1-r)$	59
			Pm4 pm4	$\frac{1}{2}(1-2r+2r^2)$	103
			pm4 pm4	$\frac{1}{2}r(1-r)$	37
			sr21 sr21 Pm4 Pm4	$\frac{1}{4}(1-r)^2$	43
			Pm4 pm4	$\frac{1}{2}r(1-r)$	44
			pm4 pm4	$\frac{1}{4}r^2$	11
Total	1	364		1	400

Conventional Linkage Studies

(i) *Sr21 and Pm4*

Analysis of testcross and F_2 genotypic frequencies (Table 5) for the cross Federation*4/2/Yuma/8*Chancellor/3/W3591 showed that *Pm4* and *Sr21* were not inherited independently.

Testcross

$\chi^2_{1:1:1:1} = 20{\cdot}95, \quad P < 0{\cdot}001$

F_2 genotypes

$\chi^2_{1:2:1}$ *Sr21Sr21 : Sr21sr21 : sr21sr21* $= 0{\cdot}14, \quad P > 0{\cdot}90$

$\chi^2_{1:2:1}$ *Pm4Pm4 : Pm4pm4 : pm4pm4* $= 4{\cdot}50, \quad P > 0{\cdot}10$

χ^2 difference (linkage) $= 33{\cdot}67, \quad P < 0{\cdot}001$

$\chi^2_{1:2:1:2:4:2:1:2:1}$ joint segregation $= 38{\cdot}31, \quad P < 0{\cdot}001$

The expected proportions of various genotypes in terms of the recombination value *r* are listed in Table 5. From the testcross results *r* was estimated to be $38{\cdot}8 \pm 2{\cdot}6\%$

whereas from the F_2 classifications r was estimated at $36{\cdot}5 \pm 2{\cdot}3\%$ by the method of maximum likelihood. Since the estimates were not significantly different the two sets of data were pooled to derive a single recombination value (Mather 1963) of $37{\cdot}5 \pm 1{\cdot}7\%$.

(ii) *Pm4 and Mle*

The results of three crosses for testing linkage of *Pm4* and *Mle* are summarized in Table 6. The first cross involved a TP229 derivative, the second a W804 derivative and the third included *Sr21* as a genetic marker. Since these involved crosses of heterozygous parents 25% of F_1 plants were expected to inherit a resistance allele from each parent but these would be indistinguishable from the 50% obtaining a resistance allele from either parent. As expected 25% of F_1 plants were susceptible.

F_2 tests were performed to distinguish those hybrids segregating for a single factor from those segregating for two factors. Fifteen hybrids segregated in ratios indicative of single-gene segregation whereas nine lines were non-segregating. A total of 617 seedlings were derived from non-segregating lines and 196 of these were progeny-tested in F_3. *Pm4* and *Mle* were closely linked or allelic.

Table 6. Summary of F_1, F_2 and F_3 results for testing allelism of *Pm4* and *Mle* when heterozygous plants were intercrossed and progenies tested with *E. graminis tritici* culture S.U.1

R, resistant; S, susceptible

Cross	F_1 numbers R : S	No. of F_2 families	F_2 status (pooled ratio)	No. of F_3 lines non-segregating
1[A]	6 : 2	1	Non-segregating (32 : 0)	27
		5	Segregating (108 : 32)	
2[B]	7 : 3	2	Non-segregating (46 : 0)	44
		5	Segregating (210 : 47)	
3[C]	11 : 3	6	Non-segregating (541 : 0)	125
		5	Segregating (86 : 47)	
Total	24 : 8	9	Non-segregating (617 : 0)	196
		15	Segregating (404 : 125)	

[A] TP229/6*Federation/3/Yuma/8*Chancellor/2/4*Federation.
[B] Federation*8/W804/3/Khapli/8*Chancellor/2/6*Federation.
[C] Federation*8/W804/3/*T. monococcum* W10/3**T. turgidum*/2/Steinwedel.

Forty-five of the F_3 lines from crosses 1 and 2 were tested at Cambridge with culture W76/95. All seedlings in all lines were resistant. When several lines were tested with culture N76/139 on benzimidazole–agar, segregation for *Mle* was apparent. On the other hand the same lines were susceptible with culture W76/163. These results were consistent with the expectation that populations were segregating for *Pm4* and *Mle*. In cross 3, 43 seedlings (in 16 F_3 lines) among a total of 2895 tested at the first seedling leaf stage showed mildew levels that exceeded either parent. However, no plant was as susceptible as Federation controls. When several plants with higher reactions were transplanted they were mildew resistant at later growth stages. In another experiment 13 lines (each of 40 seedlings) in which such plants had been recorded were inoculated with an *Sr21*-avirulent culture of *P. graminis tritici* 8 days prior to inoculation with *E. graminis tritici*. In this experiment only one seedling

was susceptible. This plant was totally susceptible and may have been a contaminant. As expected F_3 lines were segregating for *Sr21*.

On the assumption that no recombinant genotypes were identified in F_3 then if *Pm4* and *Mle* represent different loci the maximum recombination value r can be calculated for a nominated probability level. In the present linkage situation where F_3 lines with populations of up to 60 seedlings were tested and where test cultures of the pathogen were avirulent for the genes concerned, the recombinant products that will be recognized are *Pm4 Mle/pm4 mle* with frequency $\frac{1}{2}r^2$, *Pm4 mle/pm4 mle* and *pm4 Mle/pm4 mle* each with frequency $r(1-r)$ and *pm4 mle/pm4 mle* (which should have been detected in F_2) with frequency $\frac{1}{4}r^2$. Hence at $P = 0{\cdot}05$ the maximum value of r is given by the expression

$$[1-(r-\tfrac{1}{4}r^2)]^{196} = 0{\cdot}05$$
$$r = 0{\cdot}015$$

Discussion

Gene *Sr21* transferred from five widely different stocks of *T. monococcum* to hexaploid wheat was located in chromosome 2A. Preliminary genetic analyses of diploid wheat indicated possible duplication of genes which behave identically in response to many cultures of *P. graminis tritici*. However, subsequent tests (The 1976) and the present investigations suggest the occurrence of only a single gene which was differentially transmitted. In crosses with Chinese Spring the hexaploid derivative W3586 produced an overall F_2 ratio of 4·3 plants resistant : 1 susceptible. But the gene(s) responsible was nevertheless located in chromosome 2A. Abnormal ratios in wheat, reported for genes on chromosomes 6B (Loegering and Sears 1963; Luig 1968) and 2B (Nyquist 1962; McIntosh and Luig 1973), appear to be caused largely by linked genes affecting relative gametic viabilities, but these effects, or the linkage values, must be dependent on the genetic background (Nyquist 1962; Luig 1968). No detailed studies of the cause of abnormal ratios in diploid wheat or W3586 have been conducted.

Sr21 was located in the long arm and showed $2{\cdot}4 \pm 0{\cdot}9\%$ recombination with the centromere. Its location in 2AL was confirmed by further study of a monotelodisomic recombinant testcross plant, and by the corresponding absence of recombination of *Sr21* in a monotelodisomic F_1 plant involving 2AS. Although the identification of the 2AL telocentric chromosome was deduced from circumstantial evidence the total results are consistent with the presumption. When genes are located close to the centromere on the basis of presumed recombination tests, their specific chromosome arm locations require independent confirmation by experiments such as the use of the opposite telocentric for the particular chromosome or the association with linked genes whose locations are already known. In the present study confirmatory tests involved the use of telocentric 2AS and linkage with *Pm4* which was located either close to the centromere in 2AS, or in 2AL. Linkage of $37{\cdot}5\%$ with *Pm4* showed clearly that the latter must be located in 2AL.

Mle was located in chromosome 2A and segregated independently of the 2A centromere when tested against the 2AL telosome. Intercrosses of lines carrying *Mle* and *Pm4* indicated that these genes were closely linked, or allelic.

Intercrosses of heterozygous parents with similar phenotypes present problems when hybrid lines fail to segregate since the observed results could come from self-

pollination of the female parent. In the first two hybrids examined in Table 6, F_3 lines were shown to segregate for *Mle* when infected with an *E. graminis tritici* culture virulent for *Pm4*. The third cross segregated for *Sr21* which was present in the male parent. Hence the populations must have been of hybrid origin.

In cross 3 there were added difficulties when a low proportion of F_3 plants showed mildew levels exceeding those of the parents but less than those of Federation controls. However, it was concluded that the majority of such plants were resistant and did not result from genetic recombination. Some plants could have been contaminants since in experiments of this type where the identification of recombinants is dependent on the occurrence of recessive alleles it is difficult to establish with certainty the origin of such plants. On the other hand the availability of pathogen cultures virulent on seedlings carrying the individual genes permits the identification of unique genotypes combining the respective resistance alleles. Such cultures were unavailable in Australia. Moreover, had such cultures been available cross 3 could have enabled a linkage study of both *Pm4* and *Mle* with *Sr21*.

On the basis of present results it is proposed that *Pm4* be redesignated as *Pm4a* and that *Mle* be redesignated as *Pm4b*. Both genes are present in tetraploid wheats. Khapli emmer certainly possesses genes for mildew resistance in addition to *Pm4a* but it is unknown if additional genes are present in the Persian group of tetraploid wheats.

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