

The position on chromosome 5B of wheat of the locus determining crossability with rye

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(Received 22 January 1973)

SUMMARY

The allele kr_1 , conditioning ready crossability between wheat and rye when wheat is the female parent, is at a locus already known to be on chromosome 5B of wheat. This locus was mapped using a telocentric chromosome consisting of the long arm of chromosome 5B. Mapping was carried out by analyses of F_2 and testcross progenies. The two experiments gave a mean recombination frequency, between the locus and the centromere, of $11.45 \pm 3.0\%$. The possibility of different recombination frequencies in male and female meiosis is discussed.

1. INTRODUCTION

In common wheat, *Triticum aestivum*, a genetic system exists conditioning the crossability between wheat and rye, when wheat is the seed parent. Lein (1943) made a detailed analysis and concluded that alleles at two loci were responsible for the differences between readily and poorly crossable wheat varieties. It was suggested that the readily crossable parent had the genotype $kr_1kr_1kr_2kr_2$, whereas the poorly crossable varieties were $Kr_1Kr_1Kr_2Kr_2$. From experiments with a wheat parent that showed an intermediate level of crossability, Lein concluded that the presence of Kr_1 resulted in a more marked reduction in crossability than the presence of Kr_2 .

By using intervarietal substitution lines – in which each pair of chromosomes of the readily crossing variety Chinese Spring was, in turn, replaced by its homologue from the poorly crossing variety Hope – Riley & Chapman (1967) showed that Kr_1 and Kr_2 are located on chromosomes 5B and 5A respectively. Kr_1 is probably on the long arm of chromosome 5B (Riley & Chapman, unpublished).

Lein (1943) as well as Riley & Chapman (1967) found the genotypes for ready crossability in material of Chinese origin. Tozu (1966) has reported that five Japanese wheat varieties had high crossability. The difference between high and low crossability in the Japanese material was not caused by differences in pollen tube growth but probably by disturbances just before or at fertilization.

Sears (1962, 1966) has described methods of linkage mapping in wheat using telocentrics. With these methods it is possible to position a locus on a chromosome

in terms of the arm on which it lies and to determine its crossover distance from the centromere. In the present work the long arm telocentric chromosome $5B^L$ was used to estimate the crossover distance between the gene Kr_1 and the centromere of chromosome 5B.

2. MATERIAL AND METHODS

Two parental types were used: first an intervarietal substitution line in which the pair of 5B chromosomes of the variety Chinese Spring was replaced by the pair of homologues from the variety Hope. This line will be designated CS/Hope 5B; it has a poor crossability with rye (Riley & Chapman, 1967). Secondly a line of Chinese Spring was used in which chromosome 5B was represented by a pair of telocentrics for the long arm; the short arm being entirely absent. This line will be designated CSDT $5B^L$, its crossability with rye is expected to be the same as the euploid Chinese Spring, which has high crossability. Both lines were produced by Dr E. R. Sears (Columbia, Missouri, U.S.A.). Euploid plants of the wheat varieties Chinese Spring and Hope were also used as control lines.

Chromosome numbers and the telocentric or complete status of chromosome 5B were checked in root-tip squashes. Roots of germinated seed were pre-treated in a α -monobromonaphthalene solution, fixed in glacial acetic acid, hydrolyzed in normal hydrochloric acid, stained with leuco-basic fuchsin and the tips squashed in 45% acetic acid, according to well-known methods.

Crossability of the wheat plants was tested by pollinating with rye and recording the seed set. Emasculation and pollination were carried out by experienced workers (five persons in total) each working with his own favourite method. Twenty emasculated florets were pollinated, with abundant pollen of rye, in each spike. Care was taken that the stigmas of the florets were feathery and receptive and that the rye anthers were about to dehisce. Two spikes of each wheat plant were used in the crosses. To remove as much as possible the variation due to workers, each of the two spikes of each plant was emasculated and pollinated by a different worker. All the crosses of an experiment were usually carried out by only two workers. The rye pollen parent was always the variety Petkus.

3. DESCRIPTION OF THE EXPERIMENTS

The cross CS/Hope 5B \times CSDT $5B^L$ was made to produce F_1 plants that had 20 normal pairs of chromosomes, all from the variety Chinese Spring, and had 5B heteromorphic with one complete chromosome from Hope and one long arm telocentric from Chinese Spring. The F_1 was expected to be heterozygous at the locus on $5B^L$ determining crossability. Four types of gamete would be produced by these F_1 plants, differing from each other in the status of chromosome 5B and in the allele of the crossability locus present. The four types of gamete expected were: (a) complete-5B/ Kr_1 , (b) complete-5B/ kr_1 , (c) telo- $5B^L$ / Kr_1 and (d) telo- $5B^L$ / kr_1 . The (b) and (c) types would originate by recombination between the crossability locus and the centromere, the others being the parental types.

Two experiments were carried out to determine the recombination value (see Fig. 1). Three F_1 plants were used to produce F_2 progenies. The F_2 progenies contained three groups of plants according to the status of chromosome 5B. These

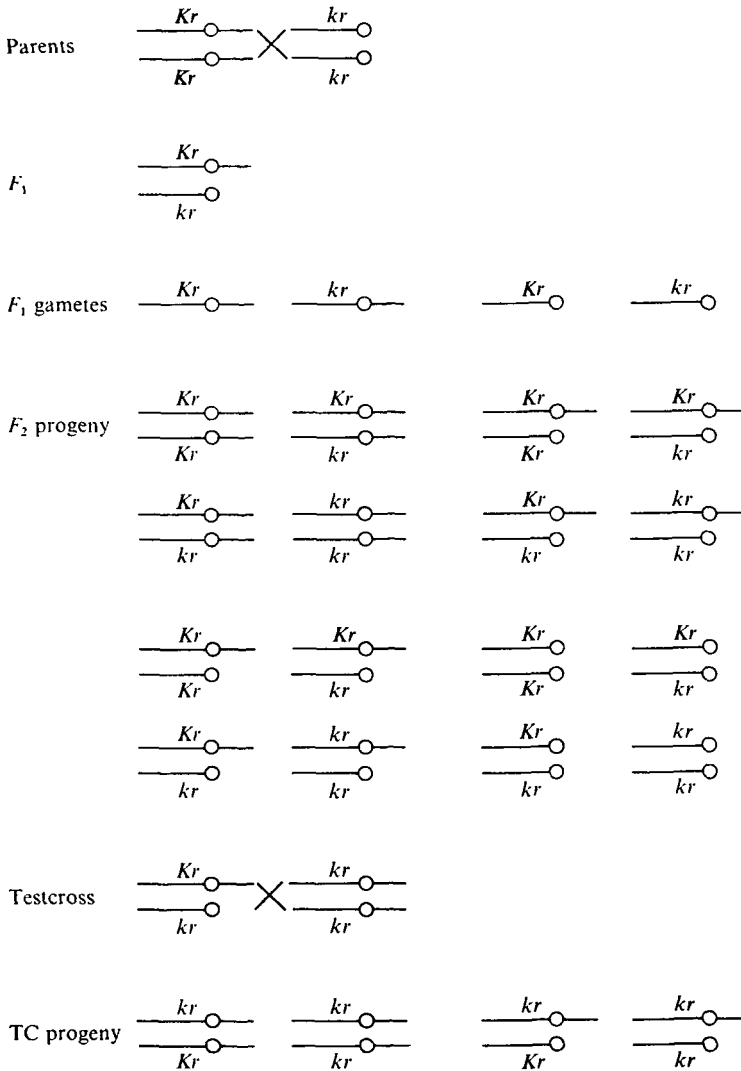


Fig. 1. Diagram illustrating the design of the experiments. The figures only represent the types of plants that can occur, in terms of cytological status of chromosome 5B of wheat and the crossability genotype, but they do not refer to the frequencies in which the types of plants occur. For the sake of clearness the gene symbols are given without subscripts.

were complete-5B/complete-5B, complete-5B/telo-5B^L and telo-5B^L/telo-5B^L, the size of the classes being dependent on the relative transmission of the complete and telocentric chromosomes. Within the classes three genotypes would occur differing in the allelic state at the crossability locus: Kr_1Kr_1 , Kr_1kr_1 and kr_1kr_1 .

The heterozygote would be phenotypically indistinguishable from the homozygous dominant. From the frequencies of these phenotypes within each cytological class, differing in the status of the 5B pair, the recombination value could be calculated.

In the second experiment five F_1 plants were used to make a testcross, in which euploid Chinese Spring, genotypically kr_1kr_1 and disomic complete-5B, was the male parent. The testcross progenies each contained two groups of plants according to the status of chromosome 5B: complete-5B/complete-5B and complete-5B/telo-5B^L. The relative size of these groups depended on the transmission of the telocentric chromosome. Within the groups two phenotypically distinguishable genotypes would occur: Kr_1kr_1 and kr_1kr_1 . Recombination would be detected by a change from the parental association of 5B status with a crossability allele.

Thus in both experiments it was necessary to establish the number of chromosomes in the plants, and the status of chromosome 5B, and then to test crossability with rye. The F_2 analysis was carried out in 1969 in Cambridge (when W. Lange was a research fellow). The testcross analysis was done in 1970. The material was divided into two parts, one part being treated in Cambridge and the other in Wageningen. In all experiments five types of plant were used as control lines: the parental lines CS/Hope 5B and CSDT 5B^L, the F_1 from CS/Hope 5B \times CSDT 5B^L, euploid Chinese Spring and euploid Hope.

4. RESULTS OF CYTOLOGICAL ANALYSIS

The cytological analysis of F_2 progeny and testcross progeny was a necessity for the experiment. From the results the gametic transmissions of the telocentric chromosome could be calculated as well.

(i) *The F_2 progeny*

The chromosomal status was determined of plants in F_2 progenies derived from three F_1 plants from the cross CS/Hope 5B \times CSDT 5B^L. In 291 F_2 plants the following numbers were scored: 145 plants were disomic for 5B complete, 135 had one complete 5B and one telocentric 5B^L, and 11 plants were disomic for the 5B^L telocentric.

If x and y are the proportions of female and male gametes carrying a telocentric chromosome, F_2 plants in the three cytological classes will be expected in the proportions:

$$\begin{array}{ll} \text{complete/complete} & (1-x)(1-y), \\ \text{complete/telocentric} & x(1-y) + y(1-x), \\ \text{telocentric/telocentric} & xy. \end{array}$$

In this way it can be calculated that the gametic transmissions of the telocentric chromosomes were $45 \pm 8\%$ and $9 \pm 9\%$. The first figure presumably represents the female transmission and the second the male transmission.

(ii) *The testcross progeny*

The chromosome status was scored of testcross progenies derived from the crosses (CS/Hope 5B \times CSDT 5B^L) \times CS euploid. There were 37 plants disomic for 5B complete and 45 with one 5B complete and one 5B^L telocentric. From these figures it could be calculated that the female transmission of the telocentric chromosome had been $54 \pm 16\%$, which does not differ significantly from the transmission found in the F_2 analysis.

5. RESULTS OF CROSSABILITY TESTS

(i) *Introduction*

The classification of the phenotypes of wheat plants as either readily or poorly crossable with rye depends on the results of artificial crosses. Although much care was taken to reduce the chance of misclassification, the characterization of some plants remained in doubt. A variance analysis was made to determine the best way of classifying the data. For this purpose the percentage fertility was transformed to θ , using the Freeman-Tukey arc-sine transformation (Mosteller & Youtz, 1961).

The analyses of variance on the whole F_2 progeny and on the two parts of the testcross progeny were used to obtain values for the least significant difference (L.S.D., $P = 0.10$) for the respective experiments. Now the experiments were classified as follows: plants of F_2 and testcross populations were considered to be readily crossable if they had a crossability value not lower than the average crossability of the readily crossable control lines in the same experiment, less the L.S.D. value of the experiment. In the same way plants of F_2 and testcross populations were considered to be poorly crossable if their crossability value was lower than the sum of the average crossability of the poorly crossable control lines plus the L.S.D. value. Using this classification method some plants could not be classified, falling between the limits set for the two categories.

(ii) *The control lines*

The percentage of successful seed set of the control lines, when pollinated with rye, is shown in Table 1. The difference between the readily crossable and the poorly crossable lines is very clear: 65.4% versus 7.8% seed set. On the data obtained for the control lines an analysis of variance was carried out to judge the technical merits of the experiments. This revealed one inconsistency, being a significant difference between the lines of the poorly crossable controls of the 1969 experiment. As can be seen from Table 1 this difference was caused by a rather good crossability of the F_1 plants compared with the other two control lines, although the level of crossability in the F_1 remained far below that of the readily crossable controls. The phenomenon did not reappear in the experiments of 1970, and will not be considered further. For the rest the comparison of the variance analyses of the controls with those of F_2 and testcross progenies did not reveal

differences in the technical aspects of the experiments. It was therefore concluded that the data of the control lines could well be used as an aid in the classification of the other data.

Table 1. *Percentage seed set of the control lines when pollinated with rye*

(Two spikes per plant and twenty florets per spike were pollinated. The figures in parentheses refer to the average fertility in units transformed according to Freeman-Tukey.)

Lines	1970							
	1969		Cambridge		Wageningen		Total	
	Plants	Fertile (%)	Plants	Fertile (%)	Plants	Fertile (%)	Plants	Fertile (%)
Chinese Spring	5	56.5	3	69.2	6	52.1	14	57.3
CSDT 5B ^L	5	79.5	3	64.2	6	73.3	14	73.6
Average		68.0		66.7		62.7		65.4
Transformed		(56.51)		(55.26)		(51.84)		
Hope	5	2.0	2	0.0	6	1.2	13	1.4
CS/Hope 5B	4	7.5	3	10.0	6	7.1	13	7.9
F ₁	5	28.0	3	2.5	7	7.5	15	13.3
Average		12.9		4.7		5.4		7.8
Transformed		(18.61)		(11.50)		(12.79)		

Table 2. *Analysis of variance of the percentage seed set following pollination by rye of the F₂ of the cross CS/Hope 5B × CSDT 5B^L, together with the L.S.D. value (P = 0.10)*

(Data transformed according to Freeman-Tukey.)

Item	D.F.	Mean square	Variance ratio
Crossers	1	2071	21.16**
Plants	73	512.4	5.23**
Error	73	97.91	—

L.S.D. (P = 0.10) = 16.48. ** P < 0.01.

(iii) *The F₂ analysis*

Plants of three F₂ progenies were pollinated with rye and the success of the crosses observed. All double telosomic plants were used in the crosses, except for one which died before flowering. The other two cytological classes were represented in the three progenies in equal proportions, aiming at ten plants per class per progeny. The analysis of the F₂ was carried out irrespective of the cytological status of the plants.

In Table 2 the results are presented of the statistical analysis of the crossability data obtained for the F₂ progenies. The significant difference between the crossers was rather constant throughout this experiment, thus it will not disturb the final results. Plants also showed significant differences, indicating segregation for crossability.

A combination of the L.S.D. value and the values for average seed set of the

control lines in the same experiment (Table 1) made classification possible in terms of crossability. Plants with a fertility higher than $56.51 - 16.48 = 39.03$ were considered to be readily crossable and plants with a fertility value lower than $18.61 + 16.48 = 35.09$ were classified as poorly crossable. Plants with a fertility percentage between these two figures could not be classified. The results of this classification are presented in Table 3.

Table 3. *The crossability of F_2 plants of the cross CS/Hope 5B \times CSDT 5B^L when pollinated with rye*

F_2 progenies	No. of plants	Crossability		
		Ready	Poor	Inconclusive
1	33	6	25	2
2	19	2	17	0
3	22	2	18	2
Total	74	10	60	4

Table 4. *Analyses of variance of the percentage seed set following pollination by rye, of the testcross (CS/Hope 5B \times CSDT 5B^L) \times Chinese Spring, together with the L.S.D. values ($P = 0.10$)*

(Data transformed according to Freeman-Tukey.)

Experiment and item	D.F.	Mean square	Variance ratio
Cambridge			
Crossers	1	117.0	0.75 n.s.
Plants	38	494.1	3.18 **
Error	38	155.4	—
L.S.D. ($P = 0.10$) = 21.01			
Wageningen			
Crossers	1	570.8	2.64 n.s.
Plants	40	967.9	4.46 **
Error	40	216.2	—
L.S.D. ($P = 0.10$) = 24.76			

n.s. = not significant. ** = $P < 0.01$.

(iv) *The testcross analysis*

Plants of five testcross progenies were treated in the same way as the F_2 plants, half of each progeny being handled in Cambridge and the other half in Wageningen. The analyses of variance for the experiments are given in Table 4. The combination of the L.S.D. values and the values for average fertility of the control lines in the same experiment (Table 1) yielded in the Cambridge experiment a zone between the maximum value for poor crossability (32.51) and the minimum value for ready crossability (34.25), while in the Wageningen experiment a zone of overlap between these values (37.55 and 27.08) was found. Both zones were considered to represent the region of uncertainty and plants falling within them could not be classified. As can be seen from the final classification in Table 5, only two plants were not ascertained.

6. CALCULATION OF RECOMBINATION VALUES

By combining results from the determination of chromosome status and the analysis of crossability the recombination values for each group of plants can be calculated (Table 6).

Table 5. *The crossability of testcross plants of the cross (CS/Hope 5B × CSDT 5B^L) × Chinese Spring, when pollinated with rye*

Testercross progenies	No. of plants	Crossability		
		Ready	Poor	Inconclusive
1	22	10	10	2
2	7	6	1	0
3	20	11	9	0
4	18	7	11	0
5	13	9	4	0
Total	80	43	35	2

Table 6. *Combination of the results from the determination of the status of chromosome 5B and the analysis of crossability following pollination with rye, for F₂ and testcross individuals, together with the calculated recombination value between the crossability gene (Kr₁) and the centromere*

Expt	5B status	No. of plants	Crossability			Recombination (%)
			Ready	Poor	Inconcl.	
F ₂	Disomic complete	33	0	32	1	—
	Complete/telo	31	4	26	1	15.8 ± 6.7
	Ditelo	10	6	2	2	13.4 ± 12.0
Testercross	Disomic complete	37	4	31	2	11.4 ± 5.4
	Complete/telo	43	39	4	0	9.3 ± 4.4

In the F₂ the three conditions of chromosome 5B plants with ready crossability will occur in the following frequencies when r is the recombination frequency:

$$\begin{aligned} \text{complete/complete} & r^2, \\ \text{complete/telocentric} & r(1-r), \\ \text{telocentric/telocentric} & (1-r)^2. \end{aligned}$$

Each cytological class should be considered separately, thus giving three figures for the recombination value. However, as can be seen from Table 6, the recombination value was obtainable from only two of the three groups of F₂ plants. The values of 15.8 ± 6.7 and 13.4 ± 12.0 agree with each other. In the third F₂ group, disomic for 5B complete, no segregation occurred, making it impossible to calculate the recombination value.

In the testcross the frequencies of readily and poorly crossable individuals in the classes complete/complete and complete/telocentric, respectively, were a direct measure of the recombination frequency. The two values of 11.4 ± 5.4 and 9.3 ± 4.4 agree very well with each other, and are also near to the values found in the F_2 analysis.

From these values it is possible to calculate the weighted mean of the recombination frequency between the locus and the centromere, this mean being $11.45 \pm 3.0\%$.

7. DISCUSSION

Looking at the cytological analyses first, the experiments confirm the previously observed difference between female and male transmission of the telocentric chromosome. This difference is probably the result of competition between male gametes, since competition is likely to be absent or very weak between female gametes. Sears & Loegering (1968) explained differences in male transmission between the two telocentric chromosomes (long arm and short arm) of chromosome 2B of wheat, by postulating differences between the two arms in genes that are important for the functioning of the male gametes. For chromosome 2B the long arm had a much better male transmission than the short arm. However, for the telocentrics of chromosome 7A (Sears & Briggie, 1969) the short arm had the better male transmission.

Sears & Loegering (1968) suggested that the presence of telocentric chromosomes would reduce crossing-over in the proximal part of the chromosome. If this is true, the distance between the crossability locus and the centromere may actually be greater than the recombination values in Table 6.

As can be seen from Table 6 there was a difference between the results of the analyses of F_2 and testcross progenies. The weighted mean of the recombination value in the first experiment is $15.23 \pm 5.8\%$ and in the second $10.14 \pm 3.4\%$. Although the difference between the means (5.09 ± 6.77) is not statistically significant it is striking enough to require comment. The difference may have been caused by environmental conditions, since the seed used in the two experiments was produced in different years, which could have resulted in different crossing-over frequencies.

Alternatively the difference between the results of analyses of F_2 and testcross progenies may have been due to a difference in the crossing-over frequency in the female and male meiosis. Evidence from the origin of aneuploids from euploids also strongly suggests higher chiasma frequencies in female meiosis in wheat (Riley & Kimber, 1961). In the testcross only the results of crossing-over in female meiosis in the F_1 was measured, while in the F_2 the results of crossing-over in female and male meiosis are combined. It is possible to make a re-calculation of the F_2 results, based on the hypothesis of unequalness of crossing-over in female and male meiosis. If r_f and r_m are the recombination frequencies in female and male meiosis and if x and y are the fractions of functioning female and male gametes having a telo-

centric chromosome, the following formulae for the frequencies of readily crossable plants in the three cytological classes can be calculated:

$$\begin{aligned} \text{compl/compl} & r_f r_m, \\ \text{compl/telo} & \frac{x(1-y)}{x(1-y)+y(1-x)} r_m(1-r_f) + \frac{y(1-x)}{x(1-y)+y(1-x)} r_f(1-r_m), \\ \text{telo/telo} & (1-r_f)(1-r_m). \end{aligned}$$

By inserting the observed values for x and y (45% and 9% respectively), and by using the F_2 segregation ratios in the classes complete/telocentric and telocentric/telocentric (see Table 6), it can be calculated that $r_f = 11.2\%$ and $r_m = 15.5\%$. The value for r_f fits the recombination frequency found in the testcross rather well.

From this it can be concluded that the observed difference in the recombination frequency in F_2 and testcross, although not statistically significant, may well be explained by unequalness of crossing-over frequencies in female and male meiosis. In plants such differences have been reported for *Primula sinensis* (Altenburg, 1916; Gregory, de Winton & Bateson, 1923), possibly for *Pisum sativum* (de Winton, 1928) and for maize, but only for the chromosome regions proximal to the centromere (Rhoades, 1941; Burnham, 1949; Clark, 1956). These studies suggest that in general the crossing-over in the male organs is higher.

The evolutionary significance of the Kr locus has been discussed by Riley & Chapman (1967). Briefly, it seems likely that the dominant allele, the presence of which reduces crossability with rye, may have had a selective advantage in those regions where both wheat and rye grow together. Without this allele, crossing would occur and the resulting interspecific F_1 plants, being sterile, would constitute weeds generated from within the crop. Consequently genotypes of wheat with ready crossability are relict in certain Asiatic regions from which rye has been absent until recent times. Tetraploid wheats differ in the extent to which they can easily be crossed with rye, so the Kr_1 allele may have originated at this level in the polyploid series.

Now that the approximate position of the Kr_1 locus on chromosome 5B has been determined it would be valuable to determine the position of the Kr_2 locus on chromosome 5A. An assessment could then be made of the likelihood that the two loci are homoeologous, as seems probable. This would assist in the comparison of the chromosomes of homoeologous group 5 of *T. aestivum* which have many similarities in genetic activity but between which there are also curious disparities some of which may arise from the incorporation in the distal region of 5B, which includes the Ph locus (Wall, Riley & Gale, 1971), of material derived from supernumerary chromosomes of *Aegilops* (Dover & Riley, 1972).

Thanks are due to Mr V. Chapman (Cambridge) for his advice and assistance, to Mr J. Post (Wageningen) for drafting and executing the statistical analysis, and to Miss M. M. Foremniak (Cambridge) and Mr K. van Spanje (Wageningen) for making crosses. Part of this study is made possible by a post-doctorate fellowship given by Shell Research Ltd., through University College at Cambridge, to one of the authors (W.L.). This fellowship enabled him to study wheat cytogenetics at the Plant Breeding Institute, Cambridge, and is gratefully acknowledged.

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