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Genetic Studies of the Crossability of Hexaploid Wheat with Rye and *Hordeum bulbosum*

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Summary. Crosses of wheat (*Triticum aestivum* L.) Group 5 chromosome substitution lines from three different cultivar backgrounds indicate that there may be multiple alleles for reduced crossability with rye (*Secale cereale* L.) and *H. bulbosum* on both 5A and 5B chromosomes. No reduction in seed set was observed from any of the 5D substitution lines. It was found that the Kr_1 locus on chromosome 5B had a more pronounced affect on both rye and *H. bulbosum* crossability than the Kr_2 locus on chromosome 5A and that the effects of both loci were cumulative. The *Kr* alleles affected both rye and *H. bulbosum* crossability in a linear, or additive, fashion. The Kr_2 allele was shown to have little effect on *H. bulbosum* crossability compared to a significant effect on rye crossability. Tests with tetrasomic and nulli-tetrasomic lines of 'Chinese Spring' indicated that the *kr* allele is 'null' or inactive in promoting crossability while the *Kr* allele is active in reducing crossability to both rye and *H. bulbosum*. Thus, extra doses of the *kr* allele do not increase rye or *H. bulbosum* crossability in the presence of the corresponding *Kr* allele.

Key words: Crossability – Wheat – Rye – *Hordeum bulbosum*

Introduction

In barley, utilization of the *Hordeum bulbosum* Method to produce haploids appears to be feasible with any genoype, although certain backgrounds produce reduced frequencies of seeds set (Pickering and Morgan 1978). In wheat, however, the use of this technique may be restricted to a very few lines or specific genotypes (Snape et al. 1979; Falk and Kasha 1981).

In wheat the *Kr* system governing rye crossability has been shown to have an influence on *H. bulbosum* crossability (Snape et al. 1979; Falk and Kasha 1981). Correlations of $r=0.70$ and $r=0.75$ were obtained between rye and *H. bulbosum* crossability of the wheat genotypes examined in the two studies, respectively. Most of the cultivars of wheat surveyed had very low crossability to both rye and *H. bulbosum*.

The purpose of this series of studies was to examine the genetic basis in wheat of the crossability with rye and *H. bulbosum*. Variability in the effects of the genes and dosage levels of known alleles on chromosomes 5A, 5B and 5D in a 'Chinese Spring' wheat background was examined. The Group 5 chromosomes in wheat have been previously associated with control of rye crossability (Riley and Chapman 1967) and *H. bulbosum* crossability (Snape et al. 1979).

Materials and Methods

H. bulbosum clones were vernalized for 12–14 weeks in a growth chamber in an eight hour photoperiod (100 $\mu E/m^2/s$ of Photosynthetically Active Radiation) at a temperature of 5/2C (Day/Night). Vernalized plants were then transferred to the main growth room where they were grown to flowering. Wheat genotypes were sown directly into plastic pots in the main growth room which was maintained on a 17 h photoperiod (350 $\mu E/m^2/s$ PAR), 60–80% relative humidity at a temperature of 21°/17 °C (D/N). All plants were grown in a greenhouse soil mixture in 15 cm diameter pots and fertilized on a weekly basis to maintain high fertility conditions. The lighting was cool white flourescent tubes and Gro-Lux tubes supplemented with incandescent bulbs.

The wheat flowers were emasculated approximately one day prior to anthesis by clipping the top of the floret and removing the anthers with forceps. Central florets and poorly developed apical and basal spikelets were removed at the time of emasculation. Emasculated spikes were covered with glassine envelopes to prevent uncontrolled pollinations. Florets were pollinated one to three days following emasculation with fresh pollen of rye or *H. bulbosum* which was collected at midmorning in a petri dish. Pollen was applied to the stigmas with a camel hair brush. Spikes were covered with a brown paper envelope over the glassine envelope after pollination

(Pickering 1976). Spikes pollinated by *H. bulbosum* were sprayed on each of three consecutive days following pollination with approximately 0.3 ml of 75 mg/l GA_3 using an aerosol device. Data were collected as seeds set per spike at 14–16 days following pollination while total numbers of seeds and florets were used to calculate the percentage seed set.

The *H. bulbosum* pollen source was the tetraploid clone B 143, originally obtained from Dr. T. Rajhathy of the Ottawa Research Station, Ottawa, Ontario. A bulk of the pollen from the spring rye cultivars 'Blanco' and 'Gazelle' was used as the rye pollen source throughout these studies. These rye cultivars have been shown to be similar in their ability to set seed when crossed with wheat (Falk, unpublished).

Lines containing the 5A, 5B or 5D chromosomes of the cultivars 'Hope' (HP), 'Atlas 66' (AT) and 'Cheyenne' (CNN) substituted into the cultivar 'Chinese Spring' (CS) were used in crosses with rye and *H. bulbosum.* These substitution lines were chosen to evaluate variation in rye and *H. bulbosum* crossability due to genes on the Group 5 chromosomes. The Hope substitution lines were obtained from Dr. R. Riley of the Plant Breeding Institute, Cambridge, UK. and the 'Atlas 66' and 'Cheyenne' substitution lines were obtained from Dr. R. Morris of the University of Nebraska, Lincoln, Nebraska.

The hybrids of 'Chinese Spring'×CS/HP5A ($kr_1kr_1Kr_2kr_2$), 'Chinese Spring'×CS/HP5B ($Kr_1kr_1kr_2kr_2$), 'Chinese Spring'×'Marquis' ($Kr_1kr_1Kr_2kr_2$) and CS/HP5A×CS/HP5B ($Kr_1kr_1Kr_2kr_2$) and each of the parental lines, 'Chinese Spring' ($kr_1kr_1kr_2kr_2$), CS/HP5A ($kr_1kr_1Kr_2Kr_2$), CS/HP5B ($Kr_1Kr_1kr_2kr_2$) and 'Marquis' ($Kr_1Kr_1Kr_2Kr_2$) were all crossed with both rye and *H. bulbosum.* ('Marquis' is the hexaploid wheat parent of 'Hope'.) The dosage effects of each *Kr* allele were calculated and plotted.

The series of three tetrasomics and the complete series of six nullisomic-tetrasomic compensating lines for the Group 5 chromosomes in the cultivar 'Chinese Spring' wheat were all crossed with both rye and *H. bulbosum* in order to study *kr* allele dosage effects. The four F_1 Hybrids of 'Chinese Spring' and the three 'Chinese Spring' tetrasomics with the cultivar 'Marquis' were also crossed to rye and *H. bulbosum.* The 'Chinese Spring' tetrasomic and nulli-tetrasomic lines were obtained from Dr. E. R. Sears, USDA, Columbia, Missouri.

Results and Discussion

Allelic Variation

Variation in the crossability of rye and *H. bulbosum* onto 'Hope', 'Atlas 66' and 'Cheyenne' 5A, 5B and 5D chromosome substitutions into 'Chinese Spring' may provide evidence for allelic variation at the Kr loci. While recognizing that whole chromosome differences are involved and that different modifiers on these chromosomes could lead to variation in expression of Kr genes relative to crossability, the variation is more likely due to allelic differences at the Kr loci and we have interpreted our results in that way. The χ^2 analysis of such crosses gave significant cultivar, chromosome substitution and pollinator effects as well as a significant chromosome substitution×pollinator interaction. The three-way interaction was not significant and was used as the error term for the analysis of variance. Duncan's Multiple Range Test was used to determine significance groupings among the data on rye and *H. bulbosum* crossability for the three sets of substitution lines (Table 1).

The substitution lines of chromosome 5A from 'Hope', 'Atlas 66' and 'Cheyenne' into 'Chinese Spring' all have reduced rye crossability with respect to their 5D substitution lines, but only the 'Hope' 5A substitution has reduced *H. bulbosum* crossability. Substituting chromosome 5B of the three cultivars into 'Chinese Spring' reduced both rye and *H. bulbosum* crossability. However, reduction of crossability by the 'Cheyenne' 5B substitution is less than the 5B substitutions of the other two cultivars.

Based on the analysis of variance of the crossability data, there are significant interactions between the pollen parent and the substituted chromosomes. This is a result of the 5A substitutions having greater reductions in rye crossability than *H. bulbosum* crossability, while the 5B substitutions produce greater reductions in *H. bulbosum* than rye crossability. There is also a significant interaction between the source of the chromosomes (donor cultivar) and the chromosomes substituted as a result of the 'Cheyenne' 5A and 5B substitutions giving quite different results from the 'Hope' 5A and 5B substitutions. Furthermore, there are highly significant differences in crossability between the rye and *H. bulbosum* pollen parents, among chromosomes substituted (indicating different effects of genes on homoeologous chromosomes) and among cultivars donating the chromosomes (indicating the possibility of different low crossability alleles at the same locus in different cultivars).

The correlation between rye and *H. bulbosum* crossability of the substitution lines is $r = 0.91$ ($P = 0.01$). CS/HP5A has a relatively low value for both rye and *H. bulbosum* crossability compared to CS/AT5A and CS/CNN5A. CS/AT5A and CS/CNN5A are nearly equal for both rye and *H. bulbosum* crossability values, an indication that 'Atlas 66' and 'Cheyenne' probably

Table 1. Rye and *H. bulbosum* crossability (percent seed set) of wheat lines containing chromosomes 5A, 5B and 5D of cultivars 'Hope', 'Atlas 66' and 'Cheyenne' substituted into 'Chinese Spring'

Wheat cultivar	Substituted chromosome					
	5A		5B		5D	
	Rye	*H. bulb*	Rye	*H. bulb*	Rye	*H. bulb*
'Hope'	41 c[a]	28 y	11 a	4 x	92 e	50 z
'Atlas 66'	65 d	53 z	11 a	8 x	89 e	44 z
'Cheyenne'	67 d	51 z	27 b	29 y	94 e	51 z

[a] Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's Multiple Range Test

have the same crossability alleles at the Kr_2 locus on chromosome 5A and that these alleles may be different from the allele on the 'Hope' 5A chromosome.

The substitution lines of 'Hope' 5B and 'Atlas 66' 5B are similar while 'Cheyenne' 5B is somewhat higher in crossability with both rye and *H. bulbosum.* Based on this similarity, it appears that 'Hope' and 'Atlas 66' may have the same allele at the Kr_1 locus while 'Cheyenne' may have a different allele.

All three of the 5D substitution lines have nearly the same values for rye crossability as well as for *H. bulbosum* crossability (Table 1) and are similar in crossability to 'Chinese Spring' (Table 3). This lack of variation between the 5D substitution lines and 'Chinese Spring' supports earlier reports of no significant effects of substituting 5D chromosomes from other cultivars into 'Chinese Spring' (Riley and Chapman 1967), and indicates that 5D probably does not contain strong alleles influencing crossability with either rye or *H. bulbosum.* Snape et al. (1979) crossed the 21 chromosome substitution lines of 'Hope' into 'Chinese Spring' (CS/HP) with *H. bulbosum.* Of the three Group 5 chromosomes substituted from 'Hope' into 'Chinese Spring', only 5A and 5B showed reduced crossability relative to 'Chinese Spring' itself.

From the study of the 'Hope', 'Atlas 66' and 'Cheyenne' substitution lines, it appears that these cultivars may have different alleles for reduced crossability at each of the two known Kr loci on chromosomes 5A and 5B when compared to the allele in 'Chinese Spring'. 'Cheyenne' appears to have an allele on chromosome 5B which has reduced rye crossability with respect to the kr_1 allele of 'Chinese Spring', but has better crossability with rye than the Kr_1 allele which 'Atlas 66' and 'Hope' possess in common. *H. bulbosum* crossability of the 'Cheyenne' 5B substitution is also less than that of 'Chinese Spring' but greater than 'Hope' and 'Atlas 66' 5B substitution lines. The evidence presented suggests that there probably are differing alleles at both of the Kr loci and that some of these alleles may affect *H. bulbosum* crossability somewhat differently than they affect rye crossability. Krowlow (1970) has suggested that there may be allelic variation at the Kr_1 and Kr_2 loci. This study appears to confirm his speculations.

Dosage of Kr Alleles

Data on percentage seed set from the rye and *H. bulbosum* crosses onto the various Kr/kr genotypes is reported in Table 2. The χ^2 analysis indicates highly significant differences among wheat genotypes and between crosses with rye and *H. bulbosum.* The interaction is also significant and therefore precludes a valid analysis of variance. A significant rank correlation coefficient of r = 0.87 (P = 0.01) is obtained between rye and *H. bulbosum* crossability for these genotypes.

Table 2. Crossability (percent seed set) of wheat with rye and *H. bulbosum* as related to doses of *Kr* alleles

	kr_1kr_1		Kr_1kr_1		Kr_1Kr_1	
	Rye	*H. bulb*	Rye	*H. bulb*	Rye	*H. bulb*
kr_2kr_2	88	36	39	4	11	4
Kr_2kr_2	70	44	20	7	–	–
Kr_2Kr_2	41	28	–	–	2	0

The rye crossability for the various *Kr/kr* combinations is significantly correlated to Lein's (1943) rye crossability data for the same genotypes (r = 0.96, n = 7), and to Marais and Pienaar's (1977) rye crossability data (r = 0.96, n = 6). The Kr_1 and Kr_2 alleles appear to have a cumulative effect on reducing crossability as has been reported (Lein 1943; Riley and Chapman 1967; Marais and Pienaar 1977).

Multiple linear regression of the rye crossability (y) values against doses of Kr_1 (x_1) and Kr_2 (x_2) from Table 2 gives an equation of:

$$y = 75.1 - 30.7x_1 - 12.6x_2$$

with an $r^2 = 0.79$ (P = 0.05) indicating a good fit of the data to the calculated multiple linear regression line. When the regression is calculated for *H. bulbosum* crossability in relation to the doses of the *Kr* alleles (Table 2) using multiple linear regression, the equation is:

$$y = 32.7 - 17.5x_1 - 0.4x_2$$

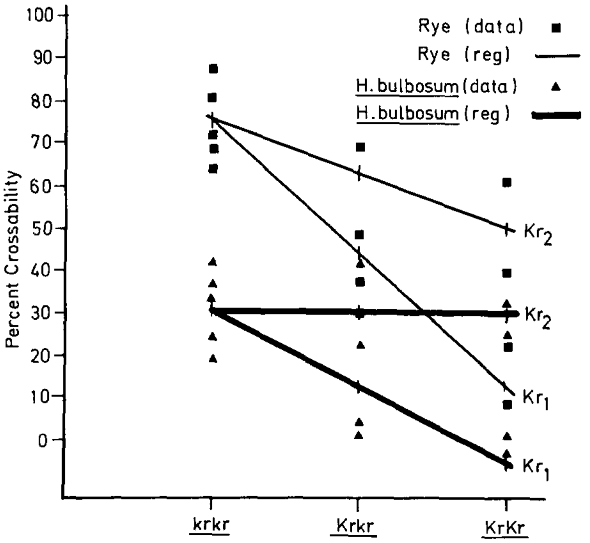

Fig. 1. Regression of rye and *H. bulbosum* crossability as related to dosage of Kr_1 and Kr_2 reduced crossability alleles

Table 3. Crossability of 'Chinese Spring' wheat tetrasomics, tetrasomics × 'Marquis' and nulli-tetrasomics with rye and *H. bulbosum*

Wheat genotype	Pollen parent			
	Rye		*H. bulbosum*	
	No. of florets	% seed set	No. of florets	% seed set
'Chinese Spring'	214	88	1376	36
CST5A	115	68	22	73
CST5B	261	59	81	35
CST5D	98	89	72	32
'Chinese Spring' × 'Marquis'	150	21	124	10
CST5A × 'Marquis'	190	17	184	0
CST5B × 'Marquis'	203	16	232	7
CST5D × 'Marquis'	352	5	208	0
CSN5AT5B	80	88	84	14
CSN5AT5D	234	24	54	13
CSN5BT5A	51	26	57	16
CSN5BT5D	102	49	78	33
CSN5DT5A	70	68	68	16
CSN5DT5B	42	43	36	78

with an $r^2 = 0.89$ ($P = 0.001$). Thus, 79% of the variation in rye crossability and 89% of the variation in *H. bulbosum* crossability can be explained by the joint action of the Kr_1 and Kr_2 alleles. The linearity of the crossability response with respect to the doses of the *Kr* alleles suggests an additive genetic system, as opposed to complete dominance of the *Kr* alleles.

Based on the regression equations, Kr_2 has little effect on *H. bulbosum* crossability (–0.4%) in comparison to the effects of Kr_1 (–17.5%). The negligible effect of Kr_2 on *H. bulbosum* crossability is in contrast to the significant effect that it has on rye crossability (–12.6%). When the regressions for both rye and *H. bulbosum* crossability are plotted against both *Kr* alleles, the relative effects of Kr_1 and Kr_2 at different dosages and with the different pollinators can be illustrated (Fig. 1).

It may be possible to breed for wheats possessing *H. bulbosum* crossability by concentrating on producing kr_1kr_1 genotypes without regard to the genotype at the Kr_2 locus. Snape and Simpson (1979) have succeeded in obtaining acceptable levels of *H. bulbosum* crossability by substituting the kr_1 allele of 'Chinese Spring' into cultivars which have low crossability. This is a time-consuming and laborious process and some method of directly overcoming crossability barriers would be preferable for a breeding program.

Dosage of kr Alleles

When the effects of extra doses of the *kr* alleles on rye and *H. bulbosum* crossability are examined (Table 3), there are significant differences between pollinators, among chromosomes and between 'Chinese Spring' and 'Marquis' cross backgrounds as indicated by χ^2 tests. All three of the two-way interactions are significant, as is the three-way interaction. Because the highest order interaction is significant, analysis of variance cannot be performed on the data. From examination of the data in Table 3, differences between rye and *H. bulbosum* pollen sources and the differences between the tetrasomic lines in the 'Chinese Spring' background and the crosses with 'Marquis' (with the introduction of Kr_1 and Kr_2) are apparent. The differences among the lines are not clearly evident as there does not seem to be a consistent pattern to the variation in rye and *H. bulbosum* crossability across lines. The three-way interaction is also an indication of some inconsistency in the data. A significant rank correlation of $r = 0.79$ ($P = 0.05$) is obtained between rye and *H. bulbosum* crossability for these data, indicating that the relationship between rye and *H. bulbosum* crossability seen in normal genotypes also occurs in the presence of extra doses of the *kr* allele.

Percentage seed set of the series of six nulli-tetrasomics for Group 5 is also reported in Table 3. Chi square analysis gives significant differences among nullisomic-tetrasomic lines, significant differences between pollen sources and a significant interaction which precludes an analysis of variance. There do not appear to be any consistent patterns in the data. The rank correlation between rye and *H. bulbosum* crossability ($r = -0.13$) is nonsignificant which indicates a lack of consistent or related variation in both rye and

H. bulbosum crossability percentages. The absence of *Kr* alleles, which are known to affect both rye and *H. bulbosum* crossability, may be the reason for the inconsistency of this correlation compared to previous data. The low seed sets in some of the crosses may be due to the inherent aneuploidy and partial sterility associated with the nulli-tetrasomic lines.

Extra doses of the *kr* alleles do not improve *H. bulbosum* or rye crossability by themselves (as in the 'Chinese Spring' tetrasomics) nor in combination with the *Kr* alleles ('Chinese Spring' tetrasomics × 'Marquis' crosses) and so the *kr* alleles would appear to be neutral in their effect on rye and *H. bulbosum* crossability. Although the nulli-tetrasomics are confounded by extra doses of homoeologous chromosomes, the lack of consistent, correlated variation in these lines further substantiates the theory that the *kr* alleles are the inactive or 'null' form (Riley and Chapman 1967). The data from the 'Chinese Spring' tetrasomics × 'Marquis' crosses also support this theory as the extra doses of *kr* do not seem to reduce the effects of the *Kr* alleles.

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Literature

Falk, D.E.; Kasha, K.J. (1981): Comparison of the crossability of rye (*S. cereale*) and *Hordeum bulbosum* onto wheat (*T. aestivum*). Can. J. Genet. Cytol. **23**, 81–88

Krowlow, K.D. (1970): Untersuchungen über die Kreuzbarkeit zwischen Weizen und Roggen. Z. Pflanzenzücht. **64**, 44–72

Lein, A. (1943): Die genetische Grundlage der Kreuzbarkeit zwischen Weizen und Roggen. Z. indukt. Abstamm. ungl. Vererb. **81**, 28–61

Marais. G.F.; Pienaar, de V. (1977): Hybridisation between wheat and rye. 1. Variations in the frequency of kernels set. Agroplantae **9**, 135–141

Pickering, R.A. (1976): Production of doubled haploid barley. Welsh Plant Breed. Stn., Abergstwyth Annu. Rep. 1976, pp. 61–63

Pickering, R.A.; Morgan, P.W. (1978): The production of doubled haploid barley. Welsh Plant Breed. Stn., Abergstwyth Annu. Rep. 1978, pp. 84–85

Riley, R.; Chapman, V. (1967): Inheritance in wheat of crossability with rye. Genet. Res. **9**, 259–267

Snape, J.W.; Chapman, V.; Moss, J.; Blanchard, C.E.; Miller, T.E. (1979): The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity **42**, 291–298

Snape, J.W.; Simpson, E. (1979): Crossability of wheat with *Hordeum bulbosum*. Annu. Rep. Plant Breed. Inst., Cambridge 1979, pp. 66

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