The effect of the crossability loci \textit{Kr}1 and \textit{Kr}2 on fertilization frequency in hexaploid wheat \texttimes maize crosses

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Summary. Dominant alleles of the \textit{Krl} and \textit{Kr}2 genes reduce the crossability of hexaploid wheat with many alien species, including rye and \textit{Hordeum bulbosum}, with \textit{Krl} having the greater effect. However, a cytological study of wheat ovaries fixed 48 h after pollination showed that the wheat genotypes 'Highbury' (\textit{Krl}, \textit{Kr}2) and 'Chinese Spring (Hope 5B)' (\textit{Krl}, \textit{kr}2) were crossable with 'Seneca 60' maize, fertilization occurring in 14.4 and 30.7\% of embryo sacs respectively. The latter figure was similar to the 29.7\% fertilization found in 'Chinese Spring' (\textit{krl}, \textit{kr}2). Most embryo sacs in which fertilization occurred contained an embryo but lacked an endosperm and where an endosperm was formed it was usually highly aberrant. All three wheat\texttimes maize combinations were karyotypically unstable and rapidly eliminated maize chromosomes to produce haploid wheat embryos.

Key words: Wheat – Maize – Crossability genes – Chromosome elimination – Haploids

Introduction

Lein (1943) identified two genes, \textit{Krl} and \textit{Kr}2, which had major effects on the crossability of hexaploid wheat (\textit{Triticum aestivum}) with rye (\textit{Secale cereale}). Dominant alleles of either gene reduced crossability, with \textit{Krl} having the greater effect.

Chromosome substitution studies have shown that wheats carrying \textit{Krl}, located on the long arm of chromosome 5B (Lange and Riley 1973; Sitch et al. 1985), have low seed set when pollinated with rye (Riley and Chapman 1967; Falk and Kasha 1981, 1983) and little or no seed set when pollinated with \textit{H. bulbosum} (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch et al. 1985). Wheats carrying \textit{Kr}2, located on the long arm of chromosome 5A (Sitch et al. 1985), show a less dramatic reduction in crossability with rye (Riley and Chapman 1967; Falk and Kasha 1981, 1983; Sitch et al. 1985) and remain crossable with \textit{H. bulbosum} (Snape et al. 1979; Falk and Kasha 1981, 1983; Sitch et al. 1985), albeit usually at a reduced frequency. \textit{Kr}1 and \textit{Kr}2 act independently since their effects are manifest in single chromosome substitution lines and wheats carrying both alleles have extremely low crossability (Lein 1943; Krolow 1970; Falk and Kasha 1983).

The \textit{Kr} genes act by inhibiting alien pollen tube growth at the base of the style and in the transmitting tract of the wheat ovary (Lein 1943; Lange and Wojciechowska 1976; Jalani and Moss 1980) and wheat\texttimes\textit{H. bulbosum} (Snape et al. 1980; Sitch 1984) crosses. Increased dosage of either \textit{Krl} or \textit{Kr}2 reduces crossability (Falk and Kasha 1983) but increased dosage of \textit{krl} or \textit{kr}2 fails to promote crossability (Riley and Chapman 1967; Falk and Kasha 1983). This suggests that \textit{Krl} and \textit{Kr}2 actively inhibit crossability and that \textit{krl} and \textit{kr}2 are null alleles.

The hexaploid wheat 'Chinese Spring' carries the recessive alleles \textit{krl} and \textit{kr}2 and is readily crossed with many alien species including maize (\textit{Zea mays}) where embryos were found in 22\% of florets (Laurie and Bennett 1986). Rapid elimination of maize chromosomes produced haploid wheat embryos, and in this respect the two crosses previously investigated resembled that between 'Chinese Spring' and \textit{H. bulbosum} (Barclay 1975). However, in the wheat\texttimes maize crosses, endosperm was either not formed or was highly aberrant.

It is of interest to determine the effect of the \textit{Kr} genes on wheat\texttimes maize crosses for two reasons. Firstly, haploid production via chromosome elimination in \textit{H. vulgare}\texttimes\textit{H. bulbosum} crosses has been widely used in barley breeding programs (Kasha and Reinbergs 1981; Snape 1982) but the exploitation of uniparental chromosome elimination in wheat\texttimes\textit{H. bulbosum} crosses for wheat haploid production has been prevented because of the presence of \textit{Krl} and \textit{Kr}2 in many cultivated wheats (Riley and Chapman 1967; Snape et al. 1979; Falk and Kasha 1981, 1983). This problem might be overcome if maize could hybridize with wheats carrying \textit{Krl}, or \textit{Krl} and \textit{Kr}2.
Secondly, it would be of great interest to transfer maize DNA, including active transposable elements, into wheat and this would be made considerably easier if karyotypically stable hybrids could be produced. The frequency of hybrid versus haploid production in *H. vulgare* × *H. bulbosum* crosses is known to be influenced by parental genotype (Simpson et al. 1980; Pickering 1983, 1984) and genes which affect the elimination of *H. bulbosum* chromosomes have been assigned to chromosomes 2 and 3 of *H. vulgare* (Ho and Kasha 1975). If maize could be hybridized with wheats carrying *Kr* genes the number of potential wheat parents which could be used in hybridization experiments would be greatly increased and this might improve the likelihood of recovering karyotypically stable hybrids.

**Materials and methods**

*a) Plant stocks*

Three genotypes of hexaploid wheat (*Triticum aestivum* L. 2n = 6x = 42 AABBDD) were selected for study.

1) ‘Chinese Spring’, homozygous for *kr1* and *kr2*.
2) ‘Chinese Spring’ (Hope 5B), a chromosome substitution line produced by E.R. Sears, University of Missouri, U.S.A, in which the ‘Chinese Spring’ 5B chromosome has been replaced by 5B from ‘Hope’, a variety showing low crossability with rye. This substitution line is therefore homozygous for *Kr1* and *Kr2*.
3) ‘Highbury’, a spring wheat cultivar homozygous for *Kr1* and *Kr2*.

These three wheats were used as female parents in crosses with the single-cross F1 hybrid sweetcorn ‘Seneca 60’ (*Zea mays* L. 2n = 20) and with the diploid rye cultivar ‘Petkus Spring’ (*Secale cereale* L. 2n = 14).

*b) Pollination methods*

Plants were grown in a heated greenhouse under continuous light. Wheat and rye plants were transferred to a 20°C growth cabinet with continuous light approximately one week prior to anthesis in the leading tiller. For each of ten plants of each wheat cultivar the first spike to emerge was emasculated one to two days prior to anthesis as described by Riley and Chapman (1967). One to two days later, when the stigmas had become feathery, they were pollinated with ‘Seneca 60’. Maize pollen was collected by allowing segments of tassel standing in water filled test-tubes to anthesis over silver foil. The tassel was tapped to release pollen which was then transferred to the wheat stigmas using a small camel hair brush. Pollinations were made within ten minutes of pollen release. The second spike to emerge was prepared similarly, but was pollinated with ‘Petkus Spring’ rye. Emerging rye anthers were picked up with fine forceps and the pollen shaken out over the wheat stigma.

*c) Light microscopy of embryo sac contents*

Ovaries were removed from the ears 24 or 48 h after pollination, fixed in 3 : 1 ethanol/acetic acid and stored at 4°C. For analysis the ovaries were rinsed in distilled water for 5 min, hydrolysed in 1N HCl at 60°C for 12 min, rinsed in distilled water and Feulgen stained for 2 h at room temperature. Ovaries were then rinsed in sulphur dioxide water for 10 min and transferred to distilled water. Embryo sac contents were dissected out in distilled water with the aid of a stereo microscope and the remaining tissue was discarded. A cover-slip, supported at one side by a second cover-slip, was placed over the specimen which was then flooded with 45% acetic acid. The unsquashed preparation was examined to determine whether a maize pollen tube had penetrated the micropyle and whether fertilization of either the egg cell or the polar nuclei had occurred. Recognising unfertilized egg cells and polar nuclei was found to be greatly aided by observing the tissue in this unsquashed state. For further analysis, such as the detection of micronuclei, the slide was flooded with 1% acetic orcein and the supporting cover-slip was removed to flatten the specimen. The number of cells in the embryo and endosperm was recorded wherever possible, all stages of mitosis being scored as one cell.

*d) Comparisons of fertilization frequency*

Analysis of variance was used to test the significance of differences in fertilization frequency after converting the data for percentage fertilization from individual spikes to angles (Snape et al. 1979).

**Results**

*a) The hybrid origin of embryos in wheat × maize crosses*

The 4C nuclear DNA contents of ‘Chinese Spring’ wheat and ‘Seneca 60’ maize are 69.3 pg and 9.84 pg (Bennett and Smith 1976; Laurie and Bennett 1985, respectively), while relative chromosome size within the respective genomes shows a 1.5 and 2 fold variation (Furuta et al. 1984; Bennett, unpublished). ‘Highbury’ and ‘Chinese Spring (Hope 5B)’ are expected to have nuclear DNA contents close to the value for ‘Chinese Spring’. Thus the smallest wheat chromosome is expected to be about twice the size of the largest maize chromosome in all three crosses. As in previous work (Laurie and Bennett 1986), zygotes at metaphase obtained from spikes fixed approximately 24 h after pollination contained the expected F1 combination of 21 large wheat chromosomes and 10 small maize chromosomes, confirming the hybrid origin of the embryos.

*b) The frequency of fertilization in wheat × maize crosses*

Examination of a total of 615 ovaries fixed 48 h after pollination showed that fertilization occurred in all three wheat genotypes (Table 1). This is illustrated in Fig. 1 which shows the overall percentage of fertilization (i.e. the percentage of embryo sacs per spike with fertilization of the egg cell, polar nuclei or both) for each plant of each genotype in conjunction with that plant’s figure for the overall percentage of fertilization with ‘Petkus Spring’ rye.

For ‘Chinese Spring (Hope 5B)’ (*Kr1, kr2*) and ‘Chinese Spring’ (*kr1, kr2*) the results were very similar.