Theor Appl Genet (2009) 119:1371–1381
DOI 10.1007/s00122-009-1141-z

ORIGINAL PAPER

Development of consistently crossable wheat genotypes for alien wheat gene transfer through fine-mapping of the *Kr1* locus

Isabelle Bertin · Lesley Fish · Tracie N. Foote · Emilie Knight · John Snape · Graham Moore

Received: 12 May 2009 / Accepted: 12 August 2009 / Published online: 30 August 2009

Abstract Breeders can force sexual hybridisation between wheat and related grass species to produce interspecific hybrids containing a dihaploid set of wheat and related chromosomes. This facilitates the introgression of desirable genes into wheat from the secondary gene pool. However, most elite European wheat varieties carry genes that suppress crossability, making the transfer of novel traits from exotic germplasm into elite wheat varieties difficult or impossible. Previous studies have identified at least five crossability loci in wheat. Here, the crossability locus with the largest effect, *Kr1* on chromosome arm 5BL, was fine-mapped by developing a series of recombinant substitution lines in which the genome of the normally non-crossable wheat variety 'Hobbit sib' carries a recombinant 5BL chromosome arm containing segments from the crossable variety 'Chinese Spring'. These recombinant lines were scored for their ability to cross with rye over four seasons. Analysis revealed at least two regions on 5BL affecting crossability, including the *Kr1* locus. However, the ability to set seed is highly dependent on prevailing environmental conditions. Typically, even crossable wheat lines exhibit little or no seed set when crossed with rye in winter, but show up to 90% seed set from similar crosses made in summer. By recombining different combinations of the two regions affecting crossability, wheat lines that consistently exhibit up to 50% seed set, whether crossed in the UK winter or summer conditions, were generated, thus creating a very important tool for increasing the efficiency of alien wheat transfer programmes.

Introduction

The genetic diversity available within bread wheat (*Triticum aestivum* L.) is very limited compared to that of its cultivated relatives such as rye (*Secale cereale* L.), barley (*Hordeum vulgare*) and diploid *Triticum*, and within the gene pools of wild (uncultivated) grass species such as *Aegilops*, *Triticum* and *Thinopyrum*. It is important for wheat breeders to be able to access the genetic diversity in the secondary gene pools of wild and cultivated relatives of wheat, to develop new varieties that yield well under a wide range of adverse conditions, and that have new loci for pest and disease resistance. Many wild species, in particular, possess very good resistance to diseases and tolerance to climatic stresses such as drought and extremes of temperature. However, successful exploitation of exotic germplasm in wheat breeding programmes is hindered by a number of barriers that need to be overcome to facilitate the breeding process. The first barrier is the ability to generate interspecific hybrids between wheat and related species; the second is the ability to retain the chromosomes of related species in the interspecific hybrid zygote during embryo development; and the final barrier is the ability to induce genetic exchange between the chromosomes of the two species even though these chromosomes are often divergent or

---

Communicated by P. Langridge.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1141-z) contains supplementary material, which is available to authorized users.

I. Bertin
Department of Genetics, Diversity and Ecophysiology of Cereals,
UMR INRA-UBP, 234, Avenue de Brézet,
63100 Clermont-Ferrand, France

L. Fish · T. N. Foote (✉) · E. Knight · J. Snape · G. Moore
Department of Crop Genetics, John Innes Centre,
Norwich Research Park, Colney, Norwich NR4 7UH, UK
e-mail: tracie.foote@bbsrc.ac.uk

even non-collinear. All these processes need to be enhanced if alien introgression is to be routinely deployed or exploited within wheat breeding programmes.

A number of studies are already underway to improve genetic exchange between wheat and related chromosomes. The *Ph1* (pairing homoeologous 1) locus is responsible for the diploid control of chromosome pairing behaviour in bread wheat (Riley and Chapman 1958), but also suppresses recombination between chromosomes of wild wheat species and their wheat homoeologues in synthetic amphiploids (Riley et al. 1959). The molecular characterisation of the *Ph1* locus (Griffiths et al. 2006; Al-Kaff et al. 2008) has led to the postulation that this locus might be regulated with drug treatment. This may provide a solution to enhance the ability to induce genetic exchange between related, but collinear, chromosomes. However, the barriers to generating stable interspecific hybrids in the first place still need to be overcome.

Most adapted wheat varieties, including all European varieties tested, contain a number of major genes and quantitative trait loci (QTLs) for interspecific incompatibility (Snape et al. 1979). This affects the ability to make crosses of wheat with most related species, for example with rye and *Hordeum bulbosum*, and thus restricts the introduction of new genes and alleles from exotic germplasm that can be used directly for alien introgression into wheat, meaning that ‘bridge varieties’, such as ‘Chinese Spring’, have to be used. These exotic wheat varieties bridge the gap between the wild species and highly improved modern varieties (Mujeeb-Kazi and Hettel 1995), but they are poorly agronomically adapted, and successful interspecific crosses made in this way are difficult to use in breeding.

The dominant alleles of the *Kr* genes prevent interspecific crossing by inhibiting pollen tube growth, which stops the pollen from fertilising the ovary (Sitch and Snape 1987). The *Kr1* gene, which has the largest effect on crossability (Lein 1943), has been mapped to the long arm of chromosome 5B (Lange and Riley 1973), and *Kr2* has been mapped to 5AL (Sitch et al. 1985). Two additional *Kr* genes have been postulated: *Kr3* on 5D (Krowlow 1970) and *Kr4* on 1A (Zheng et al. 1992). Another major crossability QTL, *Skr*, has been mapped on the short arm of 5B (Tixier et al. 1998; Lamoureux et al. 2002).

The development of detailed genetic maps and the construction of bacterial artificial chromosome (BAC) libraries from wheat genomes have led to the isolation of the first genes from hexaploid wheat including *VRN1* (Yan et al. 2003), the disease resistance genes *Lr21* (Huang et al. 2003), *Lr10* (Feuillet et al. 2003) and *Pm3b* (Yahiaoui et al. 2004), *Ph1* (Griffiths et al. 2006; Al-Kaff et al. 2008) and *Ppd1* (Beales et al. 2007) through map-based cloning. The initial aim of our crossability study, therefore, was to use positional cloning to identify a candidate gene for *Kr1* and to assess whether this information could be exploited to enhance the crossability process. An important outcome of this crossability study was the development of wheat lines that are consistently crossable whatever the environmental conditions, thus increasing the efficiency of alien wheat gene transfer programmes. In 1995, Snape et al. suggested that *Kr1* was probably located distal to the end of the region deleted in the *Ph1* mutant line, *ph1b,* which has a 70-Mb deletion on chromosome 5B (Sears 1977). In 1998, Zhang et al. used QTL mapping to estimate the location of *Kr1*, and it was found to be closely linked to the genes for hybrid necrosis (*Ne1*), winter variegation (*Vg*) and, most interestingly, *Ph1*.

In this study, single-chromosome recombinant lines are described for chromosome 5B. These were developed between the UK winter wheat variety ‘Hobbit sib’, which had the dominant (non-crossable) allele (*Kr1*), and a ‘Hobbit sib’ single-chromosome substitution line where chromosome 5BL had been substituted with chromosome 5BL from ‘Chinese Spring’, which possessed the recessive (crossable) allele (*kr1*). These recombinant lines were used to map markers on 5BL, including markers flanking the *Kr1* locus. Although the only crossability locus expected on this chromosome arm was *Kr1*, the mapping identified not one, but two regions on 5BL that had effects on crossability and/or fertility. More importantly, the development of the 5BL recombinants led to the identification of wheat lines that were consistently crossable under UK winter (artificial) as well as summer (natural) glasshouse conditions. This is particularly relevant for plant breeding in climates such as that of the UK because, although ‘Chinese Spring’ is normally regarded as being ‘crossable’, under the UK conditions, it often fails to set seed under winter crossing conditions in lit and heated glasshouses and exhibits highly variable seed set under equally variable UK summer conditions.

Materials and methods

Recombinant populations for fine-mapping

Recombinant substitution lines were developed from a series of crosses initially involving the crossable wheat variety ‘Chinese Spring’ and the non-crossable wheat variety ‘Hobbit sib’. ‘Chinese Spring’ has the standard chromosome 5B and 7B karyotype (5BS-5BL and 7BS-7BL), whereas in ‘Hobbit sib’ these chromosomes have reciprocally translocated arms, resulting in 5BL-7BL and 5BS-7BS chromosomes. Single-chromosome recombinant lines were developed for these wheat chromosomes as described by Miura et al. (1992). These were derived from crosses between ‘Hobbit sib’ and an inter-varietal chromosome substitution line 'Hobbit sib (Chinese Spring 5BL, 7BL)'. The substitution line has the standard 5BS-5BL and 7BS-7BL karyotype restored in the 'Hobbit sib' genetic background, but the 5B and 7B chromosomes have a short arm from 'Hobbit sib' and a long arm from 'Chinese Spring'. In total, 71 homozygous disomic recombinant substitution lines were generated that had recombination between the 'Hobbit sib' and 'Chinese Spring' 5BL arm. These were allowed to self-pollinate, and each of the resulting lines was assessed for crossability. All crosses were carried out under glasshouse conditions. High-pressure 400 W sodium lights were used as supplementary lighting for 16 h/day during the winter months.

Scoring for crossability

Crossability is defined here by the ability of a wheat line to set seed when crossed with rye (*Secale cereale* L. cv. Petkus Spring). It is a quantitative trait, subject to seasonal (environmental) variation, and is defined as the percentage of florets pollinated that set seed. Two random spikes from each recombinant plant were used to assess the crossability in each season. These were emasculated 1–3 days prior to anthesis and covered with glassine bags to prevent uncontrolled pollinations. Florets were pollinated with fresh rye pollen once the stigmas had become receptive, and the spikes were again covered with glassine bags. Two spikes from each recombinant plant were pollinated on two different dates to reduce environmental influences on crossability, such as insufficient light. As many as 20–30 florets were pollinated per spike. The percentage of pollinated florets to have set seed was scored 10–14 days after pollination. If the percentage of pollinated florets to have set seed was above 10–15%, the plant line was usually considered to be crossable, though seasonal variation in seed set made it important to score with reference to all other lines and parental controls. Crossability of the 71 recombinant substitution lines was first assessed in 1985. From this population, three lines scored as crossable (8-3, 17-2 and 72-1) and seven lines scored as non-crossable (1-1, 11-4, 14-3, 28-2, 83-5, 94-2 and 107-3) were selected as parents to produce F_1 heterozygotes with segmental recombination on 5BL. Crossability of these parental and F_1 recombinant lines was subsequently assessed for four consecutive growing seasons: winter 2006/07 (December/January), summer 2007 (May/June), winter 2007/08 (December/January) and summer 2008 (May/June) to assess the environmental effects on crossability. All wheat lines are available on request.

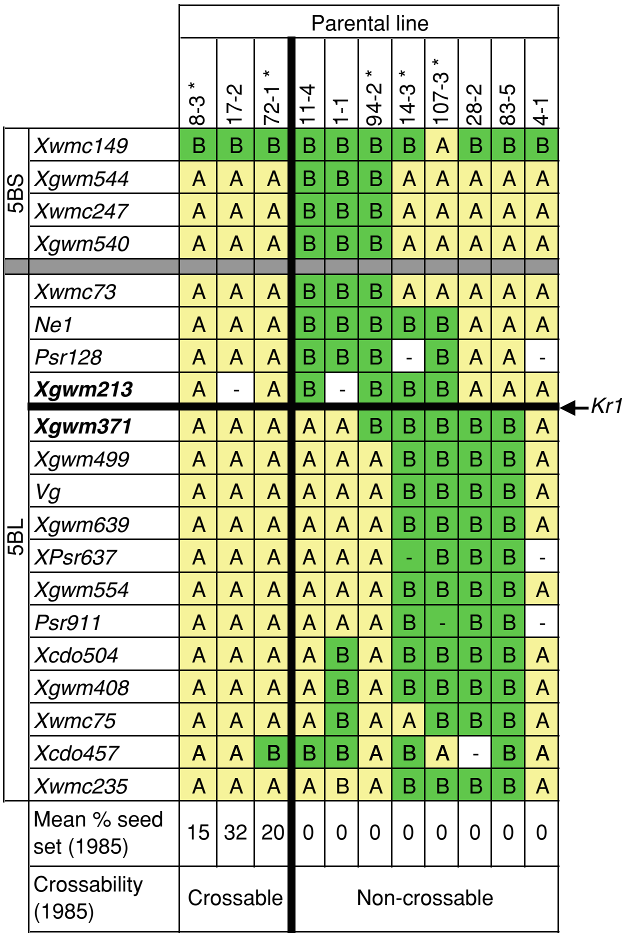
Marker development and screening of recombinant lines

Initial genotyping of the 71 recombinant lines was carried out by screening with 16 publicly available 5B-specific restriction fragment length polymorphism (RFLP) and single sequence repeat (SSR) markers distributed along the length of chromosome 5BL. Markers were derived from the published maps of Zhang et al. (1998) and Toth et al. (2003). *Gwm* primer sets were from IPK Gatersleben and *wmc* primer sets were from the Wheat Microsatellite Consortium (see GrainGenes website http://wheat.pw.usda.gov/GG2/index.shtml). This screening determined the genotype of each recombinant 5B chromosome as carrying the 'Chinese Spring' (A) or 'Hobbit sib' allele (B). Initially, the *Kr1* locus was defined to a 13-cM region between the microsatellite markers *Xgwm213* and *Xgwm371*. These markers were used to screen the *Ph1* mutant line, *ph1b*, which revealed that *Kr1* maps within the *ph1b* deletion region. Three wheat BACs (1625E21, 1275L15 and 1217B2) and the *cdc2-2* gene, located within the *Ph1* deletion region (as defined by Griffiths et al. 2006), were used to develop additional markers for mapping.

In an earlier study, wheat deletion mutants were used to fine-map the *Ph1* region, using the presence/absence of markers to delimit the locus (Roberts et al. 1999). Synteny was maintained between wheat genes within this delimited *Ph1* deletion region and genes on rice chromosome 9 (Foote et al. 1997). Knowledge of wheat–rice synteny in this region was therefore used to develop more markers to fine-map *Kr1*.

Annotated rice chromosome 9 genes in the region equivalent to the *Kr1* region (as defined by *Xgwm213* and *Xgwm371*) were used in a BLAST search of publicly available wheat and barley expressed sequence tags (ESTs). The rice coding sequence (CDS) was used to BLAST search the equivalent region in the genome of the model species *Brachypodium distachyon* using the 4x preliminary sequence assembly in the 'BrachyBase' database (http://www.brachypodium.org/). The equivalent region was found to be located on *Brachypodium* chromosome four (Bd4). The EMBOSS 'est2genome' software tool was used to aid the prediction of introns by sequence homology. This programme allowed the alignment of ESTs from the spliced nucleotide sequences with rice unspliced genomic DNA sequence. ESTs showing good homology with rice single-copy sequences were selected for marker development. Markers were then designed for displaying polymorphisms using single-strand conformational polymorphism SSCP-SNP (Bertin et al. 2005). The grass genome sequences were used to design 281 PCR primer pairs, each pair spanning an intron to take advantage of the higher variability of non-coding DNA sequences. SSCP gels were used to detect polymorphism between 'Chinese Spring' and 'Hobbit sib'.

Further markers were designed for 5BL outside the *Kr1* region because the results of the initial crossability screen suggested that there could be additional *Kr*-like loci in different locations along the 5BL chromosome arm. Synteny



		Parental line										
		8-3 *	17-2	72-1 *	11-4	1-1	94-2 *	14-3 *	107-3 *	28-2	83-5	4-1
5BS	*Xwmc149*	B	B	B	B	B	B	B	A	B	B	B
	Xgwm544	A	A	A	B	B	B	A	A	A	A	A
	Xwmc247	A	A	A	B	B	B	A	A	A	A	A
	Xgwm540	A	A	A	B	B	B	A	A	A	A	A
5BL	*Xwmc73*	A	A	A	B	B	B	A	A	A	A	A
	Ne1	A	A	A	B	B	B	B	B	A	A	A
	Psr128	A	A	A	B	B	B	-	B	A	A	-
	Xgwm213	A	-	A	B	-	B	B	B	A	A	A
	Xgwm371	A	A	A	A	A	B	B	B	B	B	A
	Xgwm499	A	A	A	A	A	A	B	B	B	B	A
	Vg	A	A	A	A	A	A	B	B	B	B	A
	Xgwm639	A	A	A	A	A	A	B	B	B	B	A
	XPsr637	A	A	A	A	A	A	-	B	B	B	-
	Xgwm554	A	A	A	A	A	A	B	B	B	B	A
	Psr911	A	A	A	A	A	A	B	-	B	B	-
	Xcdo504	A	A	A	A	B	A	B	B	B	B	A
	Xgwm408	A	A	A	A	B	A	B	B	B	B	A
	Xwmc75	A	A	A	A	B	A	A	B	B	B	A
	Xcdo457	A	A	B	B	B	A	B	A	-	B	A
	Xwmc235	A	A	A	A	B	A	B	B	B	B	A
	Mean % seed set (1985)	15	32	20	0	0	0	0	0	0	0	0
	Crossability (1985)	Crossable			Non-crossable							

Fig. 1 Graphical genotypes of recombinant parental lines mapped using chromosome 5B markers. *Eleven selected lines* are shown out of a total of 71 mapped parental lines. Crossability scores of the same lines as mean percentage seed set in summer 1985. The first ten lines were those used to make further crosses. Those marked with an *asterisk* gave progeny that were recombinant within the region of interest on chromosome 5B. The letter '*A*' (in *boxes shaded yellow*) represents the 'Chinese Spring' (*crossable*) allele (*kr1*); '*B*' (in *boxes shaded green*) represents the 'Hobbit sib' (non-crossable) allele (*Kr1*). A *dash* in an unshaded box means that no score is available

Fig. 2 Graphical genotypes and crossability scores for five parental lines and 29 homozygous segmental recombinant lines from three different crosses, mapped using chromosome 5B markers. *Xgwm*, *Xwmc* and *Xbarc* denote genomic loci (*barc* markers are from Beltsville Agricultural Research Station). The EST-SSR loci are named with the internationally assigned accession number. The letter '*A*' (in *boxes shaded yellow*) represents the 'Chinese Spring' (*crossable*) allele (*kr1*); 'B' (in *boxes shaded green*) represents the 'Hobbit sib' (*non-crossable*) allele (*Kr1*); 'H' (in *boxes shaded orange*) represents a heterozygote; '*D*' represents '*A*' or '*H*' alleles (indicating a possible weak band for '*B*'); a *dash* in an unshaded box means that no score is available and an *empty box* indicates that the marker was not used in the original scoring of the parental lines. Crossability for each parental and recombinant line with rye is shown for two to four growing seasons, scored as mean percentage seed set. The 'Hobbit sib' segment containing *Kr1* is *coloured dark green* and the region containing the *Kr1* locus is indicated by a *green bar*. The reciprocal 'Chinese Spring' segment containing *kr1* is coloured *bright yellow*. Names and crossability scores in *blue* indicate lines that are crossable despite possessing the *Kr1* non-crossable allele. The *dark blue boxes* define a region within which there is a complex interaction between the 'Chinese Spring' and Hobbit sib' alleles that causes the non-crossable lines to become partially crossable even in the presence of *Kr1*. Names and crossability scores in *red* indicate lines that are consistently crossable even when environmental conditions are sub-optimal. The 'Hobbit sib' region defined by the red box may contain alleles that interact with 'Chinese Spring' alleles on 5BL resulting in a consistently crossable phenotype. The *light blue bar* labelled "Crossability region 2" represents the 'Hobbit sib' segment in line 149-4-183 that makes this line non-crossable. The location of the *Ph1* locus is indicated by a *light pink bar* ▶

between *Brachypodium* and wheat breaks down in the distal part of 5BL (Foote et al. 1997), so in this region microsatellite markers were used instead of SSCP markers for mapping. Wheat nullisomic–tetrasomic lines (N5AT5D, N5BT5A and N5DT5B) were used to assign the markers to a specific genome.

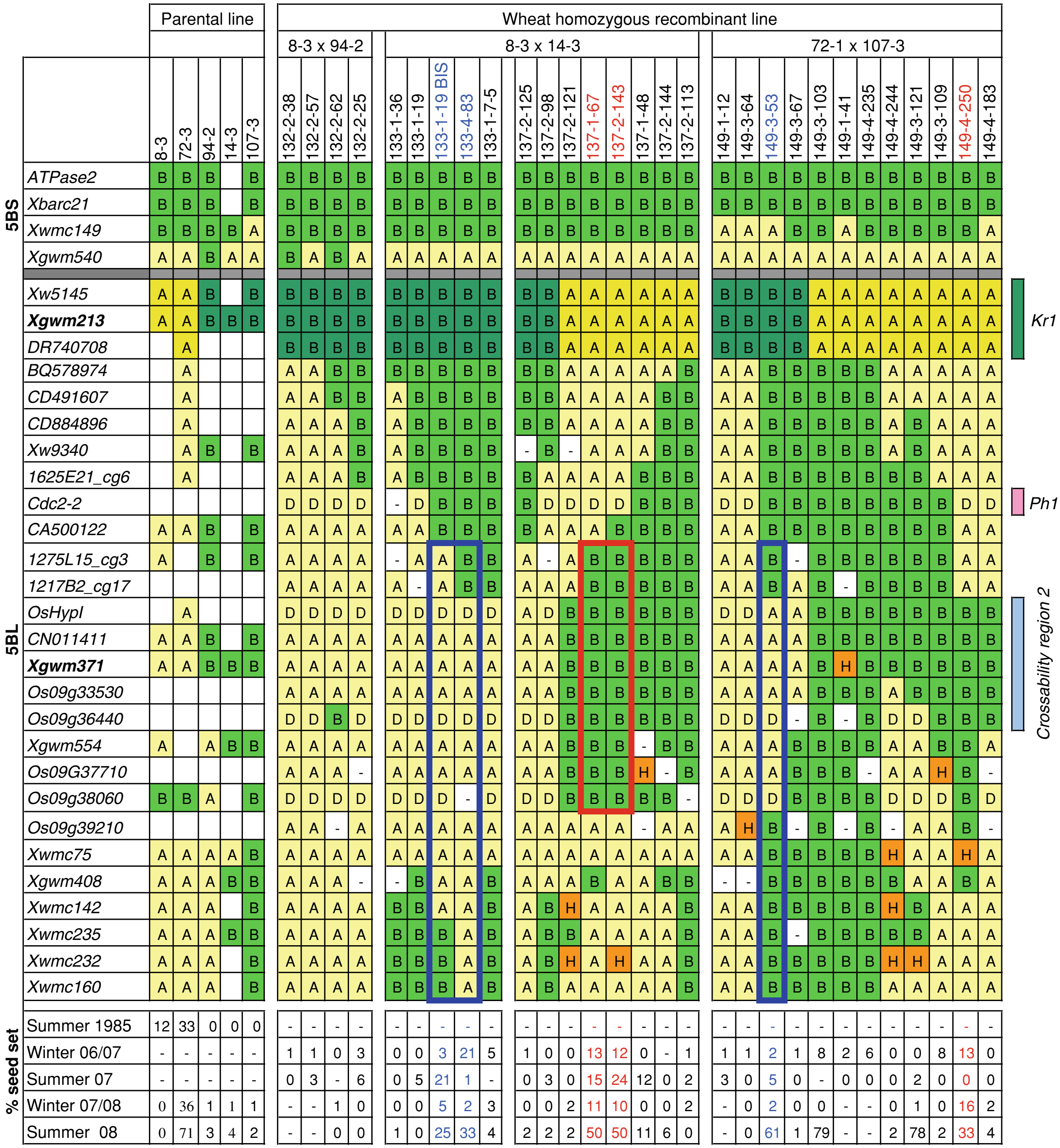
Results

Marker development

For genotype mapping, 174 primer pairs designed from ESTs were tested. Eight primer pairs yielded PCR products derived from 'Chinese Spring' and 'Hobbit sib' that were polymorphic on 5B when run on SSCP gels. In *Brachypodium distachyon,* the region equivalent to, and distal to, the *Ph1* locus in wheat was found to be located on chromosome Bd4. A total of 107 primer pairs were designed from this *Brachypodium* region. Seven of these were polymorphic on 5B. Markers used for genotyping, including wheat ESTs and *Brachypodium* markers, are shown in Supplementary Table 1.

Initial genotyping and screening for crossability

Mapping and crossability scoring of the 71 recombinant (parental) lines indicated that *Kr1* was located between the wheat microsatellite markers, *Xgwm213* and *Xgwm371*. From these 71 recombinant lines, three crossable (in these experiments) lines (8-3, 17-2 and 72-1) were further crossed with three sets of non-crossable lines (14-3, 94-2 and 107-3; 11-4, 28-2 and 107-3; 83-5, 1-1 and 107-3). The graphical genotypes and crossability scores for these selected parental lines are shown in Fig. 1. Crosses using 17-2, 11-4, 28-2 and 1-1 did not give recombination in the region of interest, but from four crosses, 137 F_1 segmental recombinant heterozygotes were identified out of 652 plant lines tested. These four crosses were from parental lines: 8-3-1 × 94-2-2, which gave rise to lines prefixed 132; 8-3-3 × 14-3-1, which gave rise to lines prefixed 133;



		Parental line					Wheat homozygous recombinant line																											
							8-3 x 94-2				8-3 x 14-3													72-1 x 107-3											
		8-3	72-3	94-2	14-3	107-3	132-2-38	132-2-57	132-2-62	132-2-25	133-1-36	133-1-19	133-1-19 BIS	133-4-83	133-1-7-5	137-2-125	137-2-98	137-2-121	137-1-67	137-2-143	137-1-48	137-2-144	137-2-113	149-1-12	149-3-64	149-3-53	149-3-67	149-3-103	149-1-41	149-4-235	149-4-244	149-3-121	149-3-109	149-4-250	149-4-183
5BS	*ATPase2*	B	B	B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Xbarc21	B	B	B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Xwmc149	B	B	B	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	B	B	A	B	B	B	B	B	A
	Xgwm540	A	A	B	A	A	B	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
5BL	*Xw5145*	A	A	B		B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	A
	Xgwm213	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	A
	DR740708		A				B	B	B	B	B	B	B	B	B	B	B	A	A	A	A	A	A	B	B	B	B	A	A	A	A	A	A	A	A
	BQ578974		A				A	A	B	B	B	B	B	B	B	B	B	A	A	A	A	A	B	A	A	B	B	B	B	B	A	A	A	A	A
	CD491607		A				A	A	B	B	A	B	B	B	B	B	B	A	A	A	A	B	B	A	A	B	B	B	B	B	A	A	A	A	A
	CD884896		A				A	A	A	B	A	B	B	B	B	B	B	A	A	A	A	B	B	A	A	B	B	B	B	B	A	B	A	A	A
	Xw9340		A	B		B	A	A	A	B	A	B	B	B	B	-	B	-	A	A	A	B	B	A	A	B	B	B	B	B	A	B	A	A	A
	1625E21_cg6		A				A	A	A	B	A	B	B	B	B	B	A	A	A	A	B	B	B	A	A	B	B	B	B	B	B	B	A	A	A
	Cdc2-2						D	D	D	D	-	D	B	B	B	B	D	D	D	D	B	B	B	D	D	B	B	B	B	B	B	B	B	D	D
	CA500122	A	A	B		B	A	A	A	A	A	A	B	B	B	B	A	A	A	B	B	B	B	A	A	B	B	B	B	B	B	B	B	A	A
	1275L15_cg3	A		B		B	A	A	A	A	-	A	A	B	B	A	-	A	B	B	B	B	B	A	A	B	-	B	B	B	B	B	B	A	A
	1217B2_cg17						A	A	A	A	A	-	A	B	B	A	A	A	B	B	B	B	B	A	A	B	A	B	-	B	B	B	B	A	A
	OsHypl		A				D	D	D	D	D	D	D	D	D	A	D	B	B	B	B	B	B	D	D	A	A	B	B	B	B	B	B	B	B
	CN011411	A	A	B		B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	B	B	B	B	B	B	B	B
	Xgwm371	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	B	H	B	B	B	B	B	B
	Os09g33530						A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B	A	A	A	A	B	B	B	A	B	B	B	B
	Os09g36440						D	D	B	D	D	D	D	D	D	D	D	B	B	B	B	B	B	D	D	D	-	B	-	B	D	D	B	B	B
	Xgwm554	A		A	B	B	A	A	A	A	A	A	A	A	A	A	A	B	B	B	-	B	B	A	A	A	B	B	B	B	A	A	B	B	A
	Os09G37710						A	A	A	-	A	A	A	A	A	A	A	B	B	B	H	-	B	A	A	A	B	B	B	-	A	A	H	B	-
	Os09g38060	B	B	A		B	D	D	D	D	D	D	D	-	D	D	D	B	B	B	B	B	-	D	D	D	B	B	B	B	D	D	D	B	D
	Os09g39210						A	A	-	A	A	A	A	A	A	A	A	A	A	A	-	A	A	A	H	B	-	B	-	B	-	A	A	B	-
	Xwmc75	A	A	A	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B	B	H	A	A	H	A
	Xgwm408	A	A	A	B	B	A	A	A	-	-	B	A	A	B	A	A	A	B	A	A	B	B	-	-	B	B	B	B	B	B	A	A	B	A
	Xwmc142	A	A	A		B	A	A	A	A	B	B	A	A	B	A	B	H	A	A	A	A	B	A	A	B	B	B	B	B	H	B	A	A	A
	Xwmc235	A	A	A	B	B	A	A	A	A	B	B	B	A	B	A	B	B	A	A	A	A	B	A	A	B	-	B	B	B	B	B	A	A	A
	Xwmc232	A	A	A		B	A	A	A	A	B	B	B	A	B	A	B	H	A	H	A	A	B	A	A	B	B	B	B	B	H	H	A	A	A
	Xwmc160	A	A	A		B	A	A	A	A	B	B	B	A	B	A	B	A	A	A	A	A	B	A	A	B	B	B	B	B	A	A	A	A	A
% seed set	Summer 1985	12	33	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Winter 06/07	-	-	-	-	-	1	1	0	3	0	0	3	21	5	1	0	0	13	12	0	-	1	1	1	2	1	8	2	6	0	0	8	13	0
	Summer 07	-	-	-	-	-	0	3	-	6	0	5	21	1	-	0	3	0	15	24	12	0	2	3	0	5	0	-	0	0	0	2	0	0	0
	Winter 07/08	0	36	1	1	1	-	-	1	0	0	0	5	2	3	0	0	2	11	10	0	0	2	-	0	2	0	0	-	-	0	1	0	16	2
	Summer 08	0	71	3	4	2	-	-	0	0	1	0	25	33	4	2	2	2	50	50	11	6	0	-	0	61	1	79	-	-	2	78	2	33	4

8-3-3 × 14-3-7, which gave rise to lines prefixed 137; and 72-1-5 × 107-3-3, which gave rise to lines prefixed 149 (Fig. 2). These were self-fertilised to generate F_2 plants, which were then genotyped using *Xgwm213* and *Xgwm371*, plus seven additional, more closely linked, molecular markers to identify recombinants in the target region (data not shown). A total of 35 F_2 plants were selected on the basis that they only recombined in the region between the markers *Xgwm213* and *Xgwm371*, thus enabling finer mapping.

From each of the 35 selected plants, 10 selfed seeds were sown and the resulting F_3 plants were genotyped using up to 31 markers (Fig. 2). Lines having no recombination between markers *Xgwm213* and *Xgwm371* were discarded. The remaining 30 lines were homozygous. Some lines that were heterozygous towards the distal end of the region were included in the study. Up to 29 homozygous lines were used in crosses with rye over four consecutive seasons from winter 2006/07 to summer 2008 (Fig. 2).

Mapping of crossability loci in parental lines

In the summer of 1985, parental lines 8-3 and 72-1 (used to generate the recombinant heterozygote population) were crossable (Fig. 1). This was consistent with the genotyping results, which indicated that the 5BL arms of these lines were derived from 'Chinese Spring'. A further 8 of the 71 recombinant lines also had a 'Chinese Spring' 5BL arm, but unlike lines 8-3 and 72-1, 3 of these, including line 4-1 (Fig. 1), did not set seed in 1985. Parental line 72-1 remained crossable in winter 2007/08 and summer 2008 (Fig. 2), but line 8-3 set no seed in these seasons, despite having been crossable in 1985.

Mapping of crossability loci in homozygous recombinant lines

Figure 3a, b shows the graphical genotypes for the five parental lines (8-3, 72-1, 94-2, 14-3 and 107-3) used in the successful crosses, and 29 homozygous segmental recombinant (progeny) lines mapped using up to 31 chromosome 5B markers. The crossability of each line with rye is shown as mean percentage seed set over two to five growing seasons. For most of the 29 homozygous recombinant lines tested that were crossable, crossability did not remain consistent over the different growing seasons, but seed set dropped to below 10% when the temperature and/or light intensity was low. These lines could therefore be termed 'partially crossable'.

Parental line 107-3, which had a 5BL arm derived from 'Hobbit sib', was non-crossable, the seed set being very low. Conversely, parental line 72-1, which had a 5BL arm derived from 'Chinese Spring', had a relatively high percentage of seed set in all three seasons that it was scored and was therefore crossable. Thus, in the homozygous recombinant population, a 5BL arm derived entirely from 'Chinese Spring' would be expected to give at least a partially crossable phenotype. However, when a segment of 'Hobbit sib' defined by markers, *Xw5145* and *DR740708*, was added to the 'Chinese Spring' arm, this resulted in most lines becoming non-crossable (Fig. 3a). Some of the recombinant lines have a larger 'Hobbit sib' segment encompassing markers *Xw5145* and *DR740708*, but the first four recombinant lines (149-1-12, 149-3-64, 133-2-38 and 133-2-57) contain the smallest segment that gives the non-crossable phenotype. So, within this 'Hobbit sib' segment defined by *Xw5145* and *DR740708*, there is a locus that has an inhibitory effect on crossability. It is likely that this segment contains the previously described *Kr1* locus.

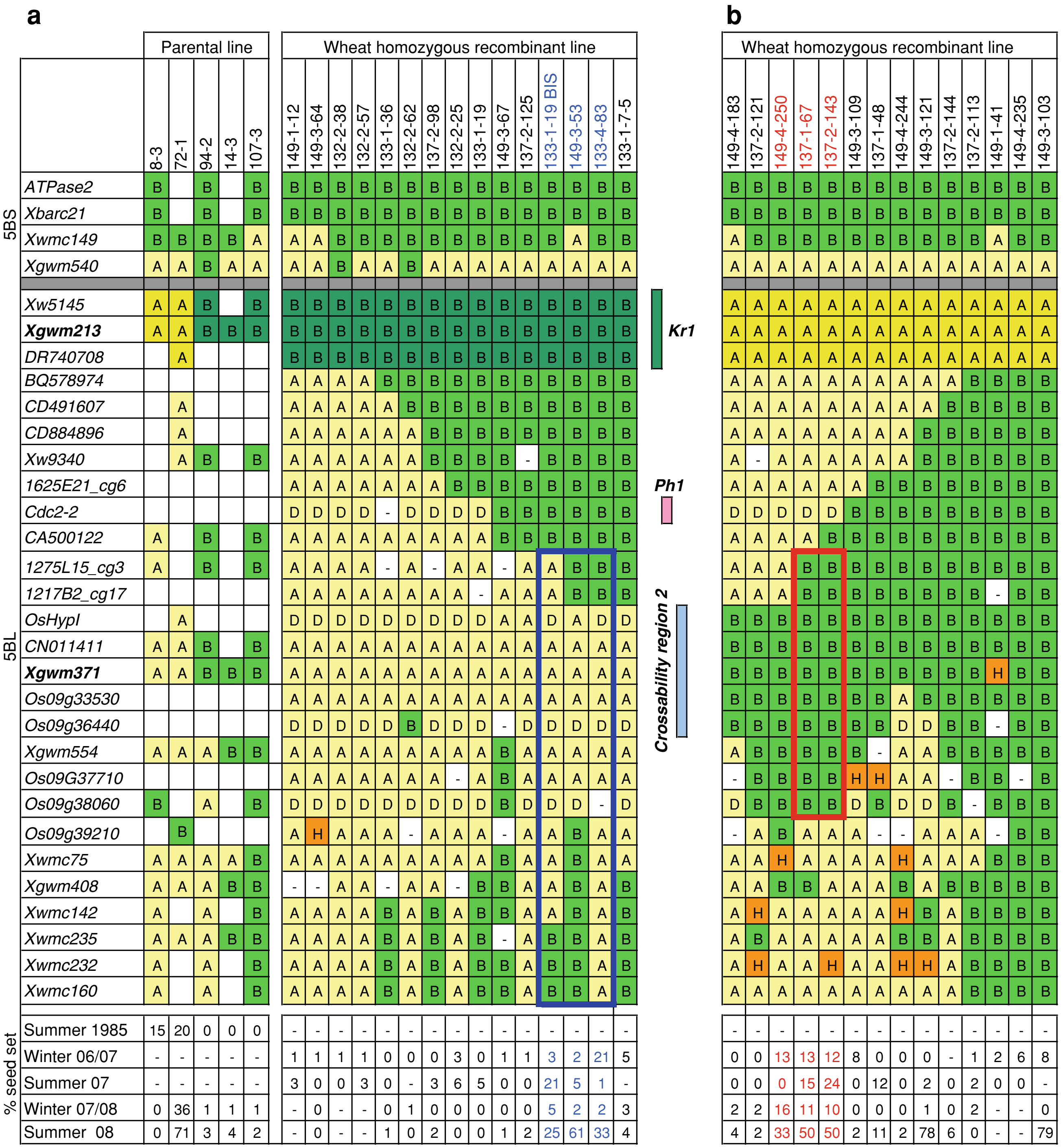
All of the recombinant lines shown in Fig. 3a carry the 'Hobbit sib' segment containing *Kr1*, and most of them are non-crossable. However, by recombining additional segments of 'Hobbit sib' from the region defined by 1275L15_cg3 and Xwmc160 (defined in Fig. 3a by a dark blue box), with the 'Chinese Spring' chromosome arm, we can produce a level of crossability even in the presence of *Kr1*. For example, the recombinant lines 133-1-19 BIS, 149-3-53 and 133-4-83 become crossable in some (though not all four) seasons. This suggests that interspecific crossability is not controlled by a single locus. There may be a complex interaction between 'Chinese Spring' and 'Hobbit sib' alleles in this region leading to partial crossability.

Fig. 3 Graphical genotypes and crossability scores for five parental lines and 29 homozygous segmental recombinant lines illustrating the effects of adding different segments of 'Chinese Spring' or 'Hobbit sib'. *Xgwm*, *Xwmc* and *Xbarc* denote genomic loci (*barc* markers are from Beltsville Agricultural Research Station). The EST-SSR loci are named with the internationally assigned accession number. The letter '*A*' (in *boxes shaded yellow*) represents the 'Chinese Spring' (*crossable*) allele (*kr1*); '*B*' (in *boxes shaded green*) represents the 'Hobbit sib' (*non-crossable*) allele (*Kr1*); '*H*' (in *boxes shaded orange*) represents a heterozygote; '*D*' represents '*A*' or '*H*' alleles (indicating a possible weak band for '*B*'); a *dash* in an unshaded box means that no score is available and an *empty box* indicates that the marker was not used in the original scoring of the parental lines. **a** All the recombinant lines carrying the 'Hobbit sib' segment containing *Kr1*. This segment is coloured *dark green*. The region containing the *Kr1* locus is indicated by a *green bar.* **b** All the recombinant lines carrying the reciprocal 'Chinese Spring' segment containing *kr1*. This segment is coloured *bright yellow*. Recombinant lines in (**a**) are non-crossable, except those where the names and crossability scores are in *blue*. The *dark blue box* defines a region within which there is a complex interaction between the 'Chinese Spring' and Hobbit sib' alleles that cause the non-crossable lines to become crossable even in the presence of *Kr1*. Recombinant lines in (**b**) are mainly non-crossable, or only crossable in good environmental conditions. Names and crossability scores in *red* indicate lines that are consistently crossable even when environmental conditions are sub-optimal. The 'Hobbit sib' region defined by the *red box* may contain alleles that interact with 'Chinese Spring' alleles on 5BL resulting in a consistently crossable phenotype. The *light blue bar* labelled "*crossability region 2*" represents the 'Hobbit sib' segment in line 149-4-183 that makes this line non-crossable. The location of the *Ph1* locus is indicated by a *light pink bar*

Generation of consistently crossable wheat genotypes

Figure 3b illustrates the effect of reciprocal recombination events on 5BL. A 5BL arm derived entirely from 'Hobbit sib' would be expected to give a non-crossable phenotype. If a segment of 'Chinese Spring' defined by the same markers, *Xw5145* and *DR740708*, is added, the lines would be expected to become crossable due to the presence of the *kr1* rather than the *Kr1* allele. Line 149-3-103, for example, has this 'Chinese Spring' *kr1* segment in a 'Hobbit sib' background and was highly crossable in summer 2008, with a seed set of 79%. However, seed set was below 10% for the two winter seasons studied, and so this crossability is inconsistent.

All the recombinant lines shown in Fig. 3b have the 'Chinese Spring' segment between markers *Xw5145* and



a

		Parental line					Wheat homozygous recombinant line														
		8-3	72-1	94-2	14-3	107-3	149-1-12	149-3-64	132-2-38	132-2-57	133-1-36	132-2-62	137-2-98	132-2-25	133-1-19	149-3-67	137-2-125	133-1-19 BIS	149-3-53	133-4-83	133-1-7-5
5BS	*ATPase2*	B		B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Xbarc21	B		B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Xwmc149	B	B	B	B	A	A	A	B	B	B	B	B	B	B	B	B	B	A	B	B
	Xgwm540	A	A	B	A	A	A	A	B	A	A	B	A	A	A	A	A	A	A	A	A
5BL	*Xw5145*	A	A	B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	Xgwm213	A	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	DR740708		A				B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
	BQ578974						A	A	A	A	B	B	B	B	B	B	B	B	B	B	B
	CD491607		A				A	A	A	A	A	B	B	B	B	B	B	B	B	B	B
	CD884896		A				A	A	A	A	A	A	B	B	B	B	B	B	B	B	B
	Xw9340		A	B		B	A	A	A	A	A	A	B	B	B	B	-	B	B	B	B
	1625E21_cg6						A	A	A	A	A	A	A	B	B	B	B	B	B	B	B
	Cdc2-2						D	D	D	D	-	D	D	D	D	B	B	B	B	B	B
	CA500122	A		B		B	A	A	A	A	A	A	A	A	A	B	B	B	B	B	B
	1275L15_cg3	A		B		B	A	A	A	A	-	A	-	A	A	-	A	A	B	B	B
	1217B2_cg17						A	A	A	A	A	A	A	A	-	A	A	A	B	B	B
	OsHypl		A				D	D	D	D	D	D	D	D	D	A	A	D	A	D	D
	CN011411	A	A	B		B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	Xgwm371	A	A	B	B	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	Os09g33530						A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
	Os09g36440						D	D	D	D	D	B	D	D	D	-	D	D	D	D	D
	Xgwm554	A	A	A	B	B	A	A	A	A	A	A	A	A	A	B	A	A	A	A	A
	Os09G37710						A	A	A	A	A	A	A	-	A	B	A	A	A	A	A
	Os09g38060	B		A		B	D	D	D	D	D	D	D	D	D	B	D	D	D	-	D
	Os09g39210		B				A	H	A	A	A	-	A	A	A	-	A	A	B	A	A
	Xwmc75	A	A	A	A	B	A	A	A	A	A	A	A	A	A	B	A	A	B	A	A
	Xgwm408	A	A	A	B	B	-	-	A	A	-	A	A	-	B	B	A	A	B	A	B
	Xwmc142	A		A		B	A	A	A	A	B	A	B	A	B	B	A	A	B	A	B
	Xwmc235	A	A	A	B	B	A	A	A	A	B	A	B	A	B	-	A	B	B	A	B
	Xwmc232	A		A		B	A	A	A	A	B	A	B	A	B	B	A	B	B	A	B
	Xwmc160	A		A		B	A	A	A	A	B	A	B	A	B	B	A	B	B	A	B
% seed set	Summer 1985	15	20	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Winter 06/07	-	-	-	-	-	1	1	1	1	0	0	0	3	0	1	1	3	2	21	5
	Summer 07	-	-	-	-	-	3	0	0	3	0	-	3	6	5	0	0	21	5	1	-
	Winter 07/08	0	36	1	1	1	-	0	-	-	0	1	0	0	0	0	0	5	2	2	3
	Summer 08	0	71	3	4	2	-	0	-	-	1	0	2	0	0	1	2	25	61	33	4

b

	Wheat homozygous recombinant line													
	149-4-183	137-2-121	149-4-250	137-1-67	137-2-143	149-3-109	137-1-48	149-4-244	149-3-121	137-2-144	137-2-113	149-1-41	149-4-235	149-3-103
ATPase2	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Xbarc21	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Xwmc149	A	B	B	B	B	B	B	B	B	B	B	A	B	B
Xgwm540	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xw5145	A	A	A	A	A	A	A	A	A	A	A	A	A	A
Xgwm213	A	A	A	A	A	A	A	A	A	A	A	A	A	A
DR740708	A	A	A	A	A	A	A	A	A	A	A	A	A	A
BQ578974	A	A	A	A	A	A	A	A	A	A	B	B	B	B
CD491607	A	A	A	A	A	A	A	A	A	B	B	B	B	B
CD884896	A	A	A	A	A	A	A	A	B	B	B	B	B	B
Xw9340	A	-	A	A	A	A	A	A	B	B	B	B	B	B
1625E21_cg6	A	A	A	A	A	A	B	B	B	B	B	B	B	B
Cdc2-2	D	D	D	D	D	B	B	B	B	B	B	B	B	B
CA500122	A	A	A	A	B	B	B	B	B	B	B	B	B	B
1275L15_cg3	A	A	A	B	B	B	B	B	B	B	B	B	B	B
1217B2_cg17	A	A	A	B	B	B	B	B	B	B	B	-	B	B
OsHypl	B	B	B	B	B	B	B	B	B	B	B	B	B	B
CN011411	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Xgwm371	B	B	B	B	B	B	B	B	B	B	B	H	B	B
Os09g33530	B	B	B	B	B	B	B	A	B	B	B	B	B	B
Os09g36440	B	B	B	B	B	B	B	D	D	B	B	-	B	B
Xgwm554	A	B	B	B	B	B	-	A	A	B	B	B	B	B
Os09G37710	-	B	B	B	B	H	H	A	A	-	B	B	-	B
Os09g38060	D	B	B	B	B	D	B	D	D	B	-	B	B	B
Os09g39210	-	A	B	A	A	A	-	-	A	A	A	-	B	B
Xwmc75	A	A	H	A	A	A	A	H	A	A	A	B	B	B
Xgwm408	A	A	B	B	A	A	A	B	A	B	B	B	B	B
Xwmc142	A	H	A	A	A	A	A	H	B	A	B	B	B	B
Xwmc235	A	B	A	A	A	A	A	B	B	A	B	B	B	B
Xwmc232	A	H	A	A	H	A	A	H	H	A	B	B	B	B
Xwmc160	A	A	A	A	A	A	A	A	A	A	B	B	B	B
Summer 1985	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Winter 06/07	0	0	13	13	12	8	0	0	0	-	1	2	6	8
Summer 07	0	0	0	15	24	0	12	0	2	0	2	0	0	-
Winter 07/08	2	2	16	11	10	0	0	0	1	0	2	-	-	0
Summer 08	4	2	33	50	50	2	11	2	78	6	0	-	-	79

DR740708. If *kr1* was the only crossability locus on this chromosome arm, we would expect all these lines to be crossable. However, some of the lines are non-crossable, which supports the hypothesis that on 5BL, there is more than one locus affecting crossability. The recombinant line 149-4-183 carries a segment of 'Hobbit sib' defined by the markers *OsHyp1* and *Os09g36440*. Although this line does not possess the *Kr1* locus, it is non-crossable, which suggests that a second *Kr* locus could lie within the region defined by this marker pair. This line is important because it has a very small 'Hobbit sib' segment (termed 'crossability region 2') compared with that of the other lines shown in Fig. 3b. So, if a second *Kr* locus was present, this line would define the locus to the smallest region. However, because this specific combination of 'Chinese Spring' and 'Hobbit sib' alleles is only possessed by line 149-4-183, further mapping is necessary to confirm the presence of a second crossability locus within this region.

More interestingly, although they possess ‘crossability region 2’, lines 137-1-67 and 137-2-143 were crossable over all four seasons in which they were studied. In these consistently crossable lines, seed set was always above 10% irrespective of low temperature and low light levels of the winter. This consistent crossability was not observed when the whole 5BL arm was composed of ‘Chinese Spring’. Line 149-4-250 gave similarly high crossability results for three seasons, though it was not crossable in summer 2007. This line was crossable in both winters and so has a similar phenotype to that of the consistently crossable lines. Indeed, it has a similar combination of ‘Chinese Spring’ and ‘Hobbit sib’ alleles as the highly crossable lines 137-1-67 and 137-2-143. Interestingly, line 149-4-250 came from a different cross to the other consistently crossable lines (Fig. 2). The fact that a similar crossability phenotype was obtained via two different crosses strengthens the case for the suggestion that this particular combination of ‘Chinese Spring’ and ‘Hobbit sib’ alleles on chromosome 5B results in wheat lines that are crossable in most winters and summers in the UK. It seems likely that there are genes on 5BL, in addition to the *Kr1* locus and crossability region 2, which affect interspecific crossability and/or fertility.

Environmental conditions during crossing seasons

Mean seasonal temperatures and mean hours of sunshine for the UK East Anglian region during the four growing seasons in this study were derived from weather statistics provided by the UK Met Office website at http://www.met-office.gov.uk/ (Supplementary Table 2). There was very little difference in mean seasonal temperature or hours of sunshine between the two winter seasons. There was no difference in mean seasonal temperature between the summer seasons in 2007 and 2008, although the summer of 1985 was slightly colder. However, there were 215 h of sunshine in summer 2008 compared with 150 h in summer 2007 and only 137 h in 1985. This may account for the generally higher seed set in the summer of 2008 (Figs. 2, 3, 4).

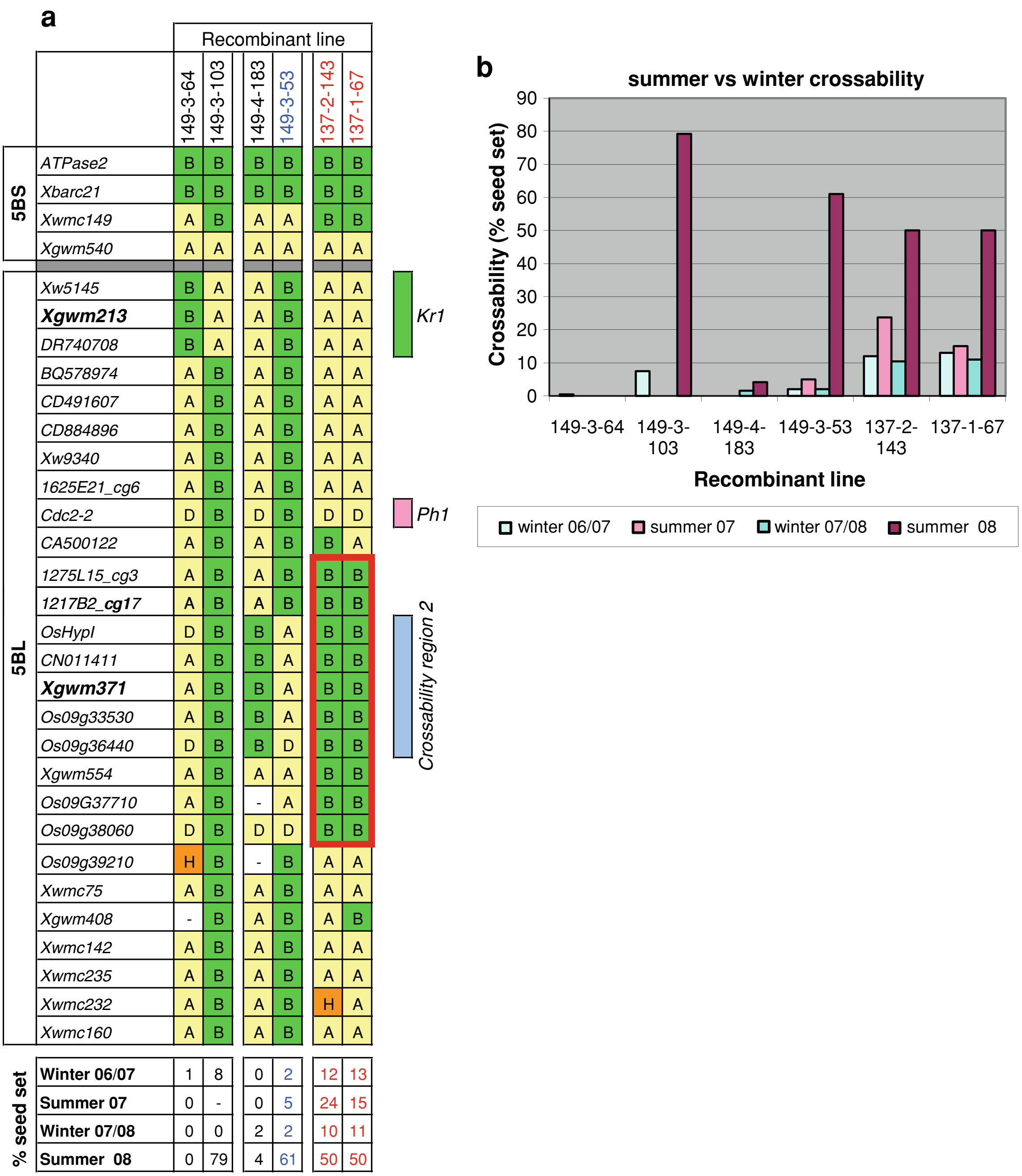
Figure 4 summarises the effects of the prevailing environmental conditions on the crossability of 6 of the 29 homozygous recombinant lines over four consecutive growing seasons. Recombinant line 149-3-64, carrying the smallest ‘Hobbit sib’ segment containing *Kr1,* and 149-4-183, carrying the ‘Hobbit sib’ segment termed crossability region 2, are non-crossable. Performing the reciprocal substitution with the corresponding ‘Chinese Spring’ chromosome segment into a ‘Hobbit sib’ 5BL chromosome can make these non-crossable wheat lines crossable. For example, line 149-3-103 carrying the *kr1* segment and 149-3-53 carrying a ‘Chinese Spring’ segment corresponding to the crossability region 2 segment were both crossable in summer 2008, with up to 79% seed set. However, 149-3-53 was not crossable in summer 2007, which had fewer hours of sunshine than 2008, and neither 149-3-53 nor 149-3-103 was crossable in UK winter conditions (no data were available for crossability of 149-3-103 in summer 2007). Lines 137-2-143 and 137-1-67 carry the same crossability region 2 ‘Hobbit sib’ segment as the non-crossable line 149-4-183, but in lines 137-2-143 and 137-1-67 this region is extended both proximally and distally. So, some factor or factors within these extended regions may have interacted with the surrounding ‘Chinese Spring’ alleles to make these lines consistently crossable.

Discussion

Detailed mapping of markers on wheat chromosome 5B has enabled *Kr1* to be located within a segment on the 5BL chromosome arm defined by the markers *Xw5145* and *DR740708* (Fig. 3a). A genetic map indicating the position of *Kr1* on 5BL is shown in Fig. 5. Our results suggest that crossability is not controlled by a single locus on 5BL, but that there is a second region on 5BL (crossability region 2) that has an effect on crossability and/or fertility, which maps just distal to the *Ph1* locus. Both this region and the *Kr1* region need further dissection to pinpoint the genes controlling crossability within them. The graphical genotypes shown in Figs. 1, 2, 3 and 4 do not necessarily reflect resolution at the molecular level and segments indicated as equivalent in these figures may have different allele combinations on the edges of the ‘identical’ segments. There may also be undetected double crossovers, which are not revealed with the markers available. Significant environmental effect on phenotype variability makes genetic analysis difficult, but a higher molecular resolution may help to resolve some of the issues. Nevertheless, it is clear that there is an interaction between the ‘Chinese Spring’ and ‘Hobbit sib’ alleles on this chromosome arm that affects crossability.

The segment defined by *Xw5145* and DR740708, containing *Kr1*, appears to be a region of low recombination. To further define the *Kr1* region, existing markers will be screened against a pooled wheat BAC library (derived from the ‘Chinese Spring’ BAC library of Allouis et al. 2003), which is under construction at the John Innes Centre, UK. Markers will be derived from the wheat BACs to build a contig map of the region, which will then be sequenced to identify candidate genes for crossability. Crossability region 2 is more amenable to further fine-mapping having a higher level of recombination and being syntenic with a region in *Brachypodium* of 2.5 Mb in size on *Brachypodium* chromosome four (Bd4).

Environmental conditions can have a profound effect on crossability. Typically, when ‘Chinese Spring’ is crossed



		149-3-64	149-3-103	149-4-183	149-3-53	137-2-143	137-1-67
5BS	*ATPase2*	B	B	B	B	B	B
	Xbarc21	B	B	B	B	B	B
	Xwmc149	A	B	A	A	B	B
	Xgwm540	A	A	A	A	A	A
5BL	*Xw5145*	B	A	A	B	A	A
	Xgwm213	B	A	A	B	A	A
	DR740708	B	A	A	B	A	A
	BQ578974	A	B	A	B	A	A
	CD491607	A	B	A	B	A	A
	CD884896	A	B	A	B	A	A
	Xw9340	A	B	A	B	A	A
	1625E21_cg6	A	B	A	B	A	A
	Cdc2-2	D	B	D	B	D	D
	CA500122	A	B	A	B	B	A
	1275L15_cg3	A	B	A	B	B	B
	*1217B2_**cg17***	A	B	A	B	B	B
	OsHypl	D	B	B	A	B	B
	CN011411	A	B	B	A	B	B
	Xgwm371	A	B	B	A	B	B
	Os09g33530	A	B	B	A	B	B
	Os09g36440	D	B	B	D	B	B
	Xgwm554	A	B	A	A	B	B
	Os09G37710	A	B	-	A	B	B
	Os09g38060	D	B	D	D	B	B
	Os09g39210	H	B	-	B	A	A
	Xwmc75	A	B	A	B	A	A
	Xgwm408	-	B	A	B	A	B
	Xwmc142	A	B	A	B	A	A
	Xwmc235	A	B	A	B	A	A
	Xwmc232	A	B	A	B	H	A
	Xwmc160	A	B	A	B	A	A
% seed set	**Winter 06/07**	1	8	0	2	12	13
	Summer 07	0	-	0	5	24	15
	Winter 07/08	0	0	2	2	10	11
	Summer 08	0	79	4	61	50	50

Fig. 4 Graphical genotypes and crossability scores for six selected homozygous segmental recombinant lines, and graphical representation of the same lines scored as mean percentage seed set over four consecutive growing seasons. **a** Graphical genotypes and crossability scores. The letter '*A*' (in *boxes shaded yellow*) represents the 'Chinese Spring' (*crossable*) allele (*kr1*); '*B*' (in *boxes shaded green*) represents the 'Hobbit sib' (*non-crossable*) allele (*Kr1*); 'H' (in *boxes shaded orange*) represents a heterozygote; '*D*' represents '*A*' or '*H*' alleles (indicating a possible weak band for '*B*'); a *dash* in an unshaded box means that no score is available. Crossability for each recombinant line with rye is shown as mean percentage seed set over four consecutive growing seasons. Recombinant line 149-3-53 is highlighted in *blue* to indicate that it is crossable despite possessing the *Kr1* non-crossable allele. Names and crossability scores in *red* indicate lines that are consistently crossable even when environmental conditions are sub-optimal. The 'Hobbit sib' region defined by the *red box* may contain alleles that interact with 'Chinese Spring' alleles on 5BL resulting in a consistently crossable phenotype. The *light blue bar* labelled "Crossability region 2" represents the 'Hobbit sib' segment in line 149-4-183 that makes this line non-crossable. The location of the *Ph1* locus is indicated by a *light pink bar*. **b** Graphical representation of summer versus winter crossability scores for the same recombinant lines. Crossability for each recombinant line with rye is shown as mean percentage seed set over four consecutive growing seasons

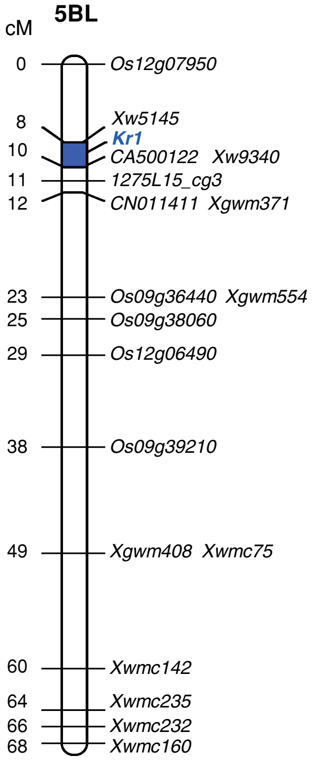


Fig. 5 Genetic map of wheat chromosome arm 5BL indicating the location of the crossability locus, *Kr1*. *Xgwm* and *Xwmc* denote genomic loci. The EST-SSR loci are named with the internationally assigned accession number. *Numbers* on the left-hand side of each map show the distances in centiMorgans between markers

with rye or *Hordeum bulbosum*, if this is carried out in a warm summer under high light levels, and the pollen is good, the seed set is high (up to around 80%). If weather conditions are bad, such as in winter in the UK, the normally crossable 'Chinese Spring' can become apparently uncrossable, with seed set possibly as low as 3%. Three parental lines with 'Chinese Spring' 5BL chromosome arms (e.g. line 4-1) did not set seed in 1985, probably due to the comparatively low temperatures and low levels of sunshine in that season (Supplementary Table 2). Parental line 8-3 *was* crossable in 1985, but set no seed in winter 2007/08 or in summer 2008 (Fig. 2), suggesting that the possession of a 'Chinese Spring' 5BL chromosome arm may confer a crossable phenotype on the line, but that this crossability is inconsistent, being highly dependant on prevailing environmental conditions. However, the introduction into a Chinese Spring 5BL arm of a segment of 'Hobbit sib', defined by the markers 1275L15_cg3 and Os09g38060, has generated wheat lines that are consistently crossable both in summer and winter seasons. These lines could have potential in breeding programmes in countries with similarly variable environmental conditions to that of the UK. Dissection of the genes conferring the consistently crossable phenotype may shed light on the regulation of fertility in wheat. The identification of wheat lines that are consistently crossable under winter or summer conditions is an important achievement in developing a robust system for exploiting alien introgression in wheat breeding programmes.

Acknowledgments This work was supported by the UK Biotechnology and Biological Sciences Research Council. *Brachypodium distachyon* sequence data were produced by the US Department of Energy Joint Genome Institute http://www.jgi.doe.gov/. The authors would also like to acknowledge Simon Griffiths for help with alignments with ESTs, Catherine Feuillet (INRA, Clermont-Ferrand, France) for the ATPase2 marker and the UK Met Office for weather statistics.

References

Al-Kaff N, Knight E, Bertin I, Foote T, Hart N, Griffiths S, Moore G (2008) Detailed dissection of the chromosomal region containing the *Ph1* locus in wheat *Triticum aestivum*: with deletion mutants and expression profiling. Ann Bot 101:863–872

Allouis S, Moore G, Bellec A, Sharp R, Faivre Rampant P, Mortimer K, Pateyron S, Foote T, Griffiths S, Caboche M, Chalhoub B (2003) Construction and characterisation of a hexaploid wheat (*Triticum aestivum* L) BAC library from the reference germplasm "Chinese Spring". Cereals Res Commun 31:331–338

Beales J, Turner A, Griffiths S, Snape JW, Laurie DA (2007) A pseudo-response regulator is misexpressed in the photoperiod-insensitive *Ppd-D1a* mutant of wheat (*Triticum aestivum* L). Theor Appl Genet 115:721–733

Bertin I, Zhu JH, Gale MD (2005) SSCP-SNP in pearl millet: a new marker system for comparative genetics. Theor Appl Genet 110:1467–1472

Feuillet C, Travella S, Stein N, Albar L, Nublat A, Keller B (2003) Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc Natl Acad Sci USA 100:15253–15258

Foote T, Roberts M, Kurata N, Sasaki T, Moore G (1997) Detailed comparative mapping of cereal chromosome regions corresponding to the *Ph1* locus in wheat. Genetics 147:801–807

Griffiths S, Sharp R, Foote TN, Bertin I, Wanous M, Reader S, Colas I, Moore G (2006) Molecular characterization of *Ph1* as a major chromosome pairing locus in polyploid wheat. Nature 439:749–752

Huang L, Brooks SA, Li W, Fellers JP, Trick HN, Gill BS (2003) Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics 164:655–664

Krowlow KD (1970) Untersuchungen uber die Kreuzbarkeit zwischen Weizen und Roggen. Z Pflanzenzucht 64:44–72

Lamoureux D, Boeuf C, Regad F, Garsmeur O, Charmet G, Sourdille P, Lagoda P, Bernard M (2002) Comparative mapping of the wheat 5B short chromosome arm distal region with rice, relative to a crossability locus. Theor Appl Genet 105:759–765

Lange W, Riley R (1973) The position on chromosome 5B of wheat of the locus determining crossability with rye. Genet Res 22:143–153

Lein A (1943) Die Genetische Grundlage der Kreutzbarkeit zwischen Weizen und Roggen. Z Induct Abstamm Vererbungsl 81:28–61

Miura H, Parker BB, Snape JW (1992) The location of major genes and associated quantitative trait loci on chromosome arm 5BL of wheat. Theor Appl Genet 85:197–204

Mujeeb-Kazi A, Hettel GP (1995) Utilizing wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT. CIMMYT Res Report 2 Mexico DF: CIMMYT

Riley R, Chapman V (1958) Genetic control of cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Riley R, Chapman V, Kimber G (1959) Genetic control of chromosome pairing in intergeneric hybrids with wheat. Nature 183:1244–1246

Roberts MA, Reader SM, Dalgliesh C, Miller TE, Foote TN, Fish LJ, Snape JW, Moore G (1999) Induction and characterisation of *Ph1* wheat mutants. Genetics 153:1909–1918

Sears ER (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Sitch LA, Snape JW (1987) Factors affecting haploid production in wheat using the *Hordeum bulbosum* system. 1. Genotypic and environmental effects on pollen germination, pollen tube growth and the frequency of fertilization. Euphytica 36:483–496

Sitch LA, Snape JW, Firman SJ (1985) Intrachromosomal mapping of crossability genes in wheat (*Triticum aestivum*). Theor Appl Genet 70:309–314

Snape JW, Chapman V, Moss J, Blanchard CE, Miller TE (1979) The crossabilities of wheat varieties with *Hordeum bulbosum*. Heredity 42:291–298

Snape J, Zhang W, Wang Y, Moore G, Foote T, Dunford R, Zheng Y (1995) Mapping of genes controlling crossability and homoeologous chromosome pairing on chromosome 5B using molecular markers. Ann Wheat Newslett 41:205–206

Tixier MH, Sourdille P, Charmet G, Gay G, Jaby C, Cadalen T, Bernard S, Nicolas P, Bernard S (1998) Detection of QTLs for crossability in wheat using a doubled-haploid population. Theor Appl Genet 97:1076–1082

Toth B, Galiba G, Fehér E, Sutka J, Snape JW (2003) Mapping genes affecting flowering time and frost resistance on chromosome 5B of wheat. Theor Appl Genet 107:509–514

Yahiaoui N, Srichumpa P, Dudler R, Keller B (2004) Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J 37:528–538

Yan L, Loukoianov A, Tranquilli G, Helguera M, Fahima T, Dubcovsky J (2003) Positional cloning of the wheat vernalization gene *VRN1*. Proc Natl Acad Sci USA 100:6263–6268

Zhang W, Zheng Y, Foote T, Doldersum J, Dunford R, Fish L, Wang Y, Moore G, Snape J (1998) Mapping key genes on chromosome 5B of wheat. In: John Innes Centre and Sainsbury Lab. Annual report 1997/98, p 12

Zheng Y, Luo M, Yen C, Yang J (1992) Chromosome location of a new crossability gene in common wheat. Wheat Info Serv 75:36–40