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Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis

1. Isolation of recombinants

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Summary. Chromosome pairing between rye chromosome arm 1RS, present in two wheat-rye translocation stocks, and its wheat homoeologues was induced by introducing the translocations into either a *ph1bph1b* or a nullisomic 5B background. This rye arm carries a gene conferring resistance to wheat stem rust, but lines carrying the translocation produce a poor quality dough unsuitable for breadmaking. Storage protein markers were utilised along with stem rust reaction to screen for allosyndetic recombinants. From a 1DL-1RS translocation, three lines involving wheat-rye recombination were recovered, along with thirteen lines derived from wheat-wheat homoeologous recombination. From a 1BL-1RS translocation, an additional three allosyndetic recombinants were recovered. Nullisomy for chromosome 5B was as efficacious as the *ph1b* mutant for induction of allosyndesis, and the former stock is easier to manipulate due to the presence of a 5BL-encoded endosperm protein. The novel wheat-rye chromosomes present in the recombinant lines may enable the rye disease resistance to be exploited without the associated dough quality defect.

Key words: Alien introgression – Cereal rye – Homoeologous recombination – *ph1b* mutant – 5B nullisomy

Introduction

Natural genetic variation within hexaploid wheat has been widely exploited by breeders, and some authors fear that it will soon be largely depleted if current practices continue (Feldman and Sears 1981). Following the demonstration that the *Ph1* gene present on chromosome 5B is largely responsible for the restriction of chromosome synapsis to homologues (Sears and Okamoto 1958; Riley and Chapman 1958), there has been much interest in the possibility of introducing useful genetic variation from the alien relatives into wheat by manipulation of the *Ph1* gene. The potential of this method for widening the gene pool available for breeders has been demonstrated using both *Aegilops* spp. (Riley et al. 1968; Dvorak 1977) and *Agropyron* spp. (Sears 1973, 1981; Liang et al. 1979; Kibirige-Sebunya and Knott 1983). However, only recently has it been shown unequivocally that chromatin from cereal rye can also be recombined with wheat in this way (Koebner and Shepherd 1985).

A number of genes controlling desirable agronomic characters are known to be located on the short arm of rye chromosome 1R. The 1BL-1RS translocation is widespread among spring and winter wheats in both Europe (Mettin et al. 1973; Zeller 1973) and Mexico and is associated both with high yielding performance across a wide range of environments (Rajaram et al. 1983) and with resistance to several foliar diseases (controlled by genes *Pm8*, *Yr9*, *Sr31* and *Lr26* – McIntosh 1983); however breeding lines and cultivars carrying this translocation have been found to produce flour with a pronounced dough quality defect (Zeller et al. 1982) which has prevented their use in the development of high yielding breadmaking varieties both in the United Kingdom (JA Blackman, personal communication) and in Australia (DJ Martin, pers. commun.). Also, the wheat cultivar 'Amigo' contains a 1AL-1RS translocation (Zeller and Fuchs 1983) and the rye arm carries a gene(s) for resistance to the greenbug *Schizaphis graminum* Rond. (Wood et al. 1974), and probably is responsible for both the powdery mildew (*Erysiphe graminis* em. Marchal f. sp. *tritici*) resistance (Lowry et al. 1981) and the stem rust resistance of this cultivar (McIntosh, cited in Zeller and Fuchs 1983).

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A spontaneous 1DL-1RS translocation involving 1RS from 'Imperial' rye in cv. 'Chinese Spring' was isolated in our laboratory (Shepherd 1973) and later it was found to carry a rye gene(s) conferring resistance to stem rust (Shepherd 1977). Since this gene is located on the same chromosome arm in 'Imperial' rye as *Sr31* from 'Petkus' rye, it is likely that the two genes are either identical or allelic. However, the 'Imperial' gene will be referred to as *SrR* until its relationship to *Sr31* has been elucidated. The translocation was transferred into a number of locally adapted genotypes by backcrossing and it was found that there was no yield disadvantage associated with the translocation in certain genetic backgrounds. A common feature with all backgrounds was, however, a pronounced weakness in the dough made from flour of the translocation homozygotes, and this quality defect has prevented the exploitation of this translocation in breadmaking wheats (Shepherd 1977; Shepherd and Singh 1984). Also, a spontaneous 1BL-1RS translocation chromosome was isolated during an attempt to substitute entire 1R of 'Imperial' rye for 1B in cv. 'Gabo'; this translocation carries *SrR* and is high yielding, but the translocation lines produce a dough which, although superior to that obtained from the 1DL-1RS lines, is still poorer than that from 'Gabo' itself (Shepherd and Singh 1984).

Zeller et al. (1982) have suggested that the dough quality defect of the 1BL-1RS translocation lines, expressed as 'sticky' dough, derives from deleterious gene(s) present on the rye arm. The more pronounced deleterious effect on dough quality associated with 1DL-1RS translocation lines would then imply that genes on 1BS are less able to counteract the negative effects of the rye genes than can genes on 1DS. Alternatively, the inferior quality of the wheat-rye translocation lines could be due more to the loss of wheat genes than to the gain of rye genes, with 1DS genes being more important for dough quality than 1BS genes. This latter hypothesis is supported by the earlier findings of Welsh and Hehn (1964) who demonstrated that 1D was by far the most important chromosome in determining dough quality when measured by the wheatmeal fermentation test (Pelshenke 1933).

Irrespective of the cause of the quality problem associated with the 1RS translocation, induction of recombination between 1RS and its wheat homoeologues would be expected to overcome it. Recombinant lines, in which the deleterious rye genes had been lost, or in which the critical wheat genes had been restored and which retained the gene for resistance to stem rust from 1RS, would provide the breeder with an alien gene for disease resistance without any attendant quality defect. In order to induce such recombination, it is necessary to introduce the alien chromatin into genotypes which lack *Ph1*. In the present work this was achieved using both the *ph1b* mutant of Sears (1977) and nullisomy for chromosome 5B. Putative recombinants were selected by screening appropriate progeny for dissociation of the markers *Gli-D1* and *Tri-D1* on chromosome arm 1DS (Singh and Shepherd 1985), and for dissociation of *Sec-1* and *SrR* on 1RS (Shewry et al. 1984a.)

Materials and methods

1 Plant materials

All lines are in a cv. 'Chinese Spring' background, unless specified otherwise.

a) Translocation lines: 1DL-1RS (Shepherd 1973); 1BL-1RS, and double translocation 1BL-1RS/1DL-1RS in cv. 'Gabo' (Shepherd, unpubl.).

b) Chromosome 5B stocks: ph1b mutant (Sears 1977); monosomic 5B (mono 5B) (Sears 1954); nullisomic 5B-tetrasomic 5A (N5BT5A), nullisomic 5B-tetrasomic 5D (N5BT5D) (Sears 1966).

c) Other cytogenetic stocks: ditelosomic 1DL (Dit 1DL) (Sears and Sears 1978); substitution line (1D) 1R (Shepherd 1973).

2 Methods

a) Electrophoresis. Unreduced endosperm protein extracts were routinely analysed by one dimensional (1D) and, in special cases, two dimensional (2D) SDS-PAGE, following the methods of Singh and Shepherd (1985), to detect the marker loci *Tri-D1, Gli-D1* and *Glu-D3* on chromosome arm 1DS, *Gli-B1* on 1BS and *Sec-1* on 1RS.

Acid PAGE was performed in vertical slabs, following the recipes of Bushuk and Zillman (1978), to separate an endosperm protein controlled by chromosome arm 5BL. Samples were extracted by incubation of a small segment of crushed endosperm overnight at 4 °C in 0.1 ml 2M urea (Shepherd 1968) with the addition of a trace of methyl green to serve as a tracking dye. The gels, of size 18 cm × 12.5 cm × 1 mm, were run at a constant voltage of 150V until the dye had travelled approximately 7.5 cm towards the cathode.

b) Cytology. Standard Feulgen methods were used to produce mitotic and meiotic chromosome preparations. Giemsa C-banding of meiotic chromosomes followed Singh and Shepherd (1984).

c) Stem rust (Puccinia graminis) inoculation. Embryos from gel-tested seeds were planted in soil in 58 × 40 × 10 cm boxes with up to 108 plants per box, and allowed to grow to the two leaf stage in the greenhouse. Rust inoculum (a mixture of Australian standard races 21 and 343) was collected from the susceptible wheat cultivar 'Halberd', mixed in a ratio of approximately 1:40 with talcum powder, and applied onto the moistened foliage of the test seedlings. The boxes were incubated in large plastic bags away from direct sunlight for 16 h. The seedlings were assessed for disease reaction after 14–21 days, depending on ambient temperatures.

3 Procedure for isolation of recombinants

In general outline, the procedure used was similar to that employed earlier to detect recombination between wheat chromosomes and the long arm of 1R (Koebner and Shepherd 1985).

3.1 Selection of a 1D/1DL-1RS translocation heterozygote, homozygous for ph1b

Plants heterozygous for the 1DL-1RS translocation were selected from an F_2 population derived from the cross *ph1b* mutant × translocation homozygote 1DL-1RS. The proteins Gli-D1 and Sec-1 were used as markers for the two chromosome arms 1DS and 1RS, respectively, and individuals bearing both these proteins were assumed to be translocation heterozygotes. Twenty such F_2 individuals were grown in pots in the greenhouse. At meiotic metaphase I the chromosomal configurations in pollen mother cells (pmcs) were analysed in an attempt to isolate homozygous *ph1b* plants. One putative *ph1b* homozygote was identified and pollinated by Dit 1DL to produce a test-cross population to be analysed for wheat-rye recombination. Dit 1DL, which lacks all 1DS marker genes, was used as the male parent in order to ensure that any recombination event in the female gamete would not be masked in the test-cross progeny.

3.2 Selection of translocation heterozygotes 1D/1DL-1RS and 1B/1BL-1RS, nullisomic for 5B

Mono 5B was crossed as female with both the 1DL-1RS and 1BL-1RS translocation lines and progeny with 2n = 41 were identified by mitotic chromosome counts in root tips. The chromosome constitution of these selections was verified at meiosis ($20^{II} + 1^{I}_{5B}$). They were then pollinated with N5BT5A or N5BT5D and unreduced protein extracts of the progeny seeds were screened by SDS-PAGE to select for translocation heterozygotes, followed by acid PAGE to allow those which were also nullisomic for 5B to be identified. The resulting plants were allowed to self-fertilise to give populations suitable for detection of wheat-rye recombination.

3.3 Screening for wheat-rye recombination

The endosperm of individual progeny seeds of the selected translocation heterozygotes were subjected to unreduced SDS-PAGE and scored for the protein bands Tri-1, Gli-D1 and Sec-1 (1DL-1RS material) and Gli-B1 and Sec-1 (1BL-1RS material). This scoring presented no difficulty. The corresponding embryo halves were then sown into boxes in ordered positions and the seedlings tested for reaction to stem rust. Plants carrying *SrR* develop only small restricted rust pustules surrounded by chlorotic tissue, and the infected leaf generally survives until normal senescence; susceptible plants develop large pustules which coalesce and early leaf death ensues. Any individual which possessed a non-parental combination of characters was selected as a putative recombinant and progeny tested to confirm the initial classification.

Results

1 Induction of allosyndetic recombination utilising the ph1b *mutant*

1.1 Identification of heterozygous 1D/1DL-1RS, ph1bph1b plants and screening for allosyndetic recombination among the test-cross progeny

Plants homozygous for *ph1b* were identified by a reduction in chiasma frequency, as discussed in a related earlier study (Koebner and Shepherd 1985). Among the 16 plants analysed, only one was positively identified as being homozygous for *ph1b*. The mean pairing of this plant at metaphase I was $1.37^{I}(0–6) + 5.37^{II}(1–8) + 13.67^{\textcircled{II}}(11–17) + 0.26^{III}(0–1) + 0.44^{IV}(0–1)$ in 27 pmcs (range in brackets). The mean chromosomal configurations of the remaining plants conformed more closely to that expected for a normal euploid as given by Driscoll et al. (1979), although many pmcs possessed a trivalent or quadrivalent. The likely origin of such multivalents in plants of genotype *Ph1b ph1b* has been discussed previously (Koebner and Shepherd 1985).

A total of 397 test-cross progeny was obtained by pollinating this plant with Dit 1DL and 394 were analysed by SDS-PAGE (Fig. 1, Table 1). Chromosome

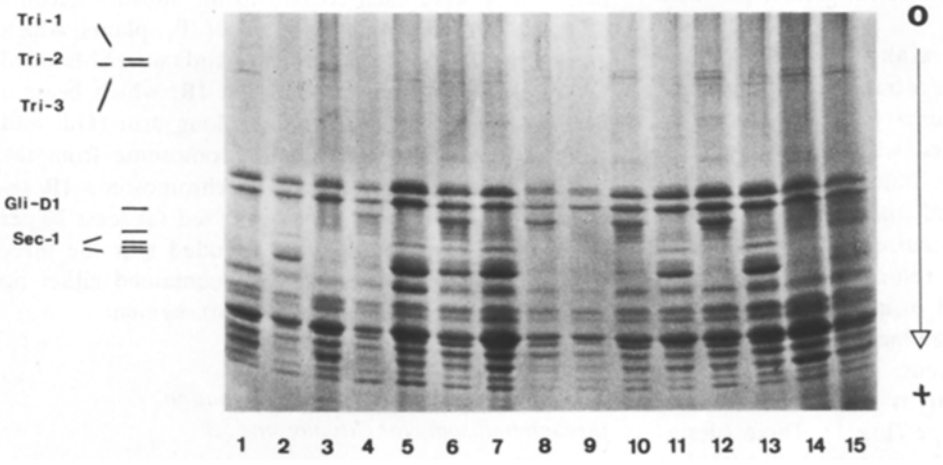

Fig. 1. Storage protein phenotypes of controls and progeny from test-crosses between 1D/1DL-1RS heterozygote *ph1bph1b* × Dit 1DL. Patterns obtained by SDS-PAGE of unreduced protein extracts. Controls: *7* Translocation 1DL-1RS (Tri-1$^-$Gli-D1$^-$Sec-1$^+$); *8* 'Chinese Spring' euploid (Tri-1$^+$Gli-D1$^+$Sec-1$^-$); *9* Dit 1DL (Tri-1$^-$Gli-D1$^-$Sec-1$^-$). Test-cross progeny: *1–6, 10–15.* Note non-parental phenotypes in *lanes 3* (plant 82-177); *6* (82–180); *14* (82–178) and *15* (82–179); *3, 14* and *15* Tri-1$^+$Gli-D1$^-$Sec-1$^-$; *6* Tri-1$^-$Gli-D1$^+$Sec-1$^+$

Table 1. Observed frequency of progeny from the test-cross 1D/1DL-1RS heterozygote, *ph1bph1b* × Dit 1DL having the endosperm protein phenotype indicated

Endosperm protein phenotype			No. observed	Classification
Tri-1	Gli-D1	Sec-1		
+	+	–	159	Parental
–	–	+	152	Parental
–	–	–	40	Hypoploid
+	+	+	39	Hyperploid
+	–	–	3	Recombinant
–	+	+	1	Recombinant

+ = protein present; – = protein absent

Table 2. Marker loci present on the recombinant chromosome in 4 plants selected from the test cross 1D/1DL-1RS heterozygote, *ph1bph1b* × Dit 1DL with respect to storage protein loci on chromosome arms 1DS and 1RS, and a rye stem rust resistance locus

Plant no.	1DS markers		1RS markers	
	Tri-D1	*Gli-D1*	*Sec-1*	*SrR*
82-177	+	–	–	–
82-178	+	–	–	–
82-179	+	–	–	–
82-180	–	+	+	+

+ = locus present; – = locus absent

arm 1DS carries three endosperm protein loci, *Tri-D1*, *Gli-D1* and *Glu-D3*. The absence of *Tri-D1* causes the loss of bands Tri-1 and Tri-2. The product of *Gli-D1* (a prolamin) appears as a single band of greater mobility than the Tri proteins. The rye gene *Sec-1* codes for a group of at least four prolamins with greater mobility than Gli-D1 (Fig. 1).

The parental phenotypes result from the transmission of either a normal 1D or a translocation chromosome 1DL-1RS from the heterozygote, and these two chromosomes were transmitted with equal frequency through the female gametes. Transmission of neither chromosome (hypoploidy) and transmission of both chromosomes (hyperploidy) occurred with almost equal frequency and 20% of the test-cross progeny were aneuploid. Such a high level of aneuploidy was predictable on the basis of the high incidence of univalents observed in the female parent. Only four putative allosyndetic recombinant embryos were detected on the basis of SDS-PAGE phenotype (Fig. 1). Three (designated 82-177, -178 and -179) possessed *Tri-D1* but neither *Gli-D1* nor *Sec-1*, while one (82-180) had lost *Tri-D1* but retained both *Gli-D1* and *Sec-1*. 2D SDS-PAGE of these four recombinants revealed that the two loci *Gli-D1* and *Glu-D3*, known to be tightly linked (Payne et al. 1984; Singh and Shepherd 1985), remained associated in all four seeds – that is, the three lacking *Gli-D1* also lacked *Glu-D3* while the seed which retained *Gli-D1* also possessed *Glu-D3*. A total of 347 of the 394 test-cross progeny analysed by SDS-PAGE produced seedlings which could be rust tested. The rust reaction showed complete linkage with the Sec-1 phenotype, that is, all progeny lacking the rye protein bands were stem rust susceptible, while those carrying these bands expressed the translocation-type resistance. The inferred genotypes of the four recombinant plants recovered in this population with respect to 1DS and 1RS storage protein loci and stem rust reaction are summarised in Table 2.

The protein phenotype of each of the four putative recombinants was confirmed by SDS-PAGE testing of their progeny obtained by self-fertilisation. In each case these progeny segregated for the recombinant protein phenotype and for a Dit 1DL phenotype, as expected since each plant had received one dose of telosome 1DL from the test-cross male parent. The Gli-D1 and Sec-1 bands always remained associated in the progeny of 82-180, indicating that the two loci controlling these bands are present on the same chromosome in this recombinant. Singh (1985) has estimated the map distance between the centromere and *Tri-D1* to be 15.4 cM, while the *Tri-D1* to *Gli-D1* distance was measured to be 45.5 cM in a separate 1DS mapping experiment (Koebner, unpubl.). This suggests that the 82-180 line possesses a recombinant chromosome made up of a proximal segment of 1RS joined to a distal segment of 1DS, with the breakpoint along 1DS occurring between the *Tri-D1* and the *Gli-D1* loci.

In an attempt to find whether the three recombinant lines 82-177, -178 and -179 contained any rye chromatin, they were each crossed to the substitution line (1D) 1R. The metaphase I pmcs of F_1 plants which carried the *Tri-D1*-coded protein bands were C-banded to search for any pairing between 1R, which bears a heterochromatic telomere on the long arm (Gill and Kimber 1974), and any other chromosome from the recombinant lines. In each case, chromosome 1R remained unpaired in all pmcs analysed (at least 20 per plant), and it was therefore concluded that the three plants 82-177, 82-178 and 82-179 contained either no rye chromatin, or at most only a short segment.

2 Induction of allosyndetic recombination through nullisomy for chromosome 5B

2.1 Use of acid PAGE to detect nullisomy for chromosome 5B

A urea soluble protein which migrates rapidly in the starch gels described by Shepherd (1968) was shown by standard

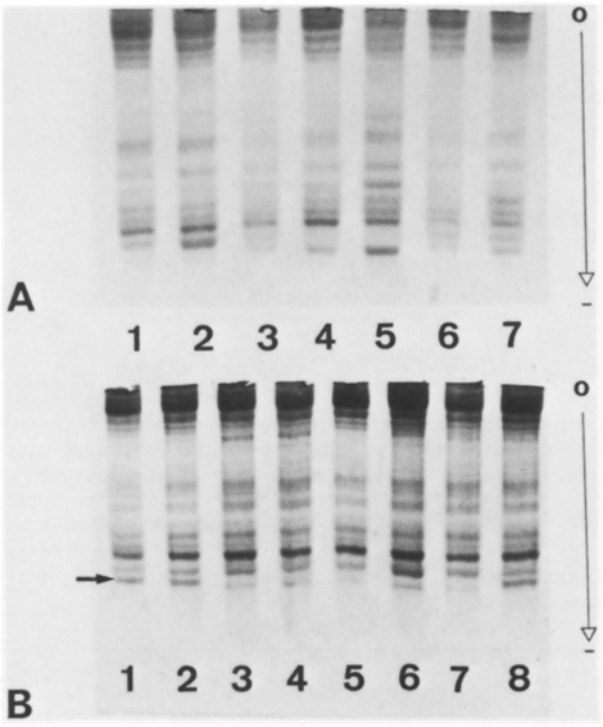

Fig. 2 A, B. Phenotypes of euploid 'Chinese Spring' (CS) and critical homoelogous group 5 aneuploids for urea soluble endosperm proteins separated by acid PAGE. **A** Homoeologous group 5 aneuploids and euploid CS: *1* N5BT5A; *2* N5BT5D; *3* Dit 5BL; *4* N5DT5A; *5* N5DT5B; *6* Dit 5DL; *7* Euploid CS. **B** Control, and progeny from the F_2 of the cross (mono 5B × translocation 1DL-1RS) × N5BT5A. Parents: *1* Euploid CS; *5* N5BT5A. Control: *6* N5BT5D. Progeny: *2, 3, 4, 7, 8.* Samples 3–7 do not carry the chromosome 5B encoded marker (*arrowed*)

aneuploid analysis to be controlled by a gene on the long arm of chromosome 5B, while another protein of slightly lower mobility is controlled by a gene on chromosome 5DL (Shepherd, unpubl.) Identical conclusions are obtained by acid PAGE separations of cv. 'Chinese Spring' aneuploids of homoeologous group 5 (Fig. 2 A). The absence of a particular band in genotypes nullisomic for 5B (lanes 1, 2) and of a different band in 5D nullisomics (lanes 4, 5), and the reinforcement of these bands in tetrasomic 5B (lane 5) and tetrasomic 5D (lane 2), respectively, support the conclusion that structural genes controlling these proteins are located on these two chromosomes. The pattern shown by the two respective long arm ditelosomic lines (lanes 3, 6) resembles that of euploid 'Chinese Spring' (lane 7) and hence these genes must be present on the long arms of these chromosomes. The absence of chromosome 5A has no observable effect on the banding pattern in these gels. A minor band with greater mobility than the 5B encoded band was observed in some gels, and was more pronounced in samples lacking the 5B band (Fig. 2 B, lanes 3–7), but the genetic control of this protein could not be elucidated. It is thought likely that the genes controlling the major bands are identical with one or other of the two independent series of homoeoloci on group 5 chromosomes coding for endosperm proteins recently described by Payne et al. (1985).

Using this procedure, the 11 Gli-D1$^+$Sec-1$^+$ individuals identified from among the 27 F_1 seeds from the cross (mono 5B × 1DL-1RS) × N5BT5A or × N5BT5D were screened for 5B nullisomy. These individuals were assumed to be 1D/1DL-1RS heterozygotes on the basis of their prolamin phenotype. Six putative nullisomic 5B seeds were identified (Fig. 2 B), and their embryos were germinated and grown in the glasshouse to produce selfed seed. One of these plants (83-39) set much more seed than any of its five sibs and it was therefore crossed to *Ae. variabilis* in order to test whether chromosome 5B was present. High levels of allosyndetic pairing occur in hybrids of wheat × *Ae. variabilis* when 5B is absent, whereas very little pairing results when *Ph1* is present (Driscoll 1968). Two of the hybrid progeny showed allosyndetic pairing in metaphase I pmcs, but one plant exhibited mainly univalents and an occasional rod bivalent and therefore plant 83-39 was inferred to be monosomic rather than nullisomic for chromosome 5B; its progeny was used as a control population for assessing the effectiveness of 5B-nullisomy for inducing allosyndetic recombination. Hybridisation between the other five putative nullisomic 5B selections and *Ae. variabilis* gave progeny which all showed allosyndetic pairing at meiotic metaphase I; however, since the expected transmission of a 5B monosome through the female gamete is only about 30% (Tsunewaki 1963), many such hybrids need to be tested to differentiate between a nulli- and a monosomic 5B plant. There were large differences in self fertility among the five presumed nullisomic 5B plants, and this was thought to result from variation in dosage of the homoeologues of 5B among these plants, with increased dosage giving partial fertility restoration (Sears 1966).

Four plants heterozygous 1B/1BL-1RS and nullisomic for 5B were selected in the same manner from among the F_1 seeds of the cross (mono 5B × 1BL-1RS) × N5BT5A, except that *Gli-B1* was used as the marker for 1BS.

2.2 Screening for allosyndetic recombination

1DL-1RS. The 544 progeny from the five plants presumed to be nullisomic for 5B and heterozygous for 1D/1DL-1RS, and 227 from the monosomic 5B control plant were analysed by SDS-PAGE. All these seeds gave scorable phenotypes, except for 13 shrivelled seeds among the nullisomic 5B progeny (Table 3). Six different phenotypes were observed among the 531 seeds derived from nullisomic 5B parents, while only three of these phenotypes were recovered in the control population.

The data obtained from the five separate nullisomic 5B plants were homogeneous ($\chi^2 = 16.03$, 12 d.f. $0.1 < P < 0.2$), and

Table 3. Endosperm protein phenotypes and their frequency in progeny from 1D/1DL-1RS heterozygotes nullisomic and monosomic for 5B

Dosage of 5B in parent	No. families		Endosperm protein phenotype						Total
		Tri-1	+	+	−	+	−	−	
		Gli-D1	+	+	−	−	+	−	
		Sec-1	−	+	+	+	+	−	
Nullisomic	5		162	249	105	10	4	1	531
Monosomic	1		67	113	47	0	0	0	227

+ = protein present; − = protein absent

therefore these have been pooled. Inferring the chromosome constitution of the progeny within the control population from their SDS-PAGE phenotype, a segregation pattern of 1.42: 2.40:1 for normal 1D homozygote: 1D/1DL-1RS heterozygote: 1DL-1RS homozygote, respectively, was observed. Since the female transmission of both normal 1D and translocation 1DL-1RS is approximately 0.5 (Table 1), then the departure from a 1:2:1 segregation ratio in the F_2 must be due to differential male transmission of the two chromosomes. Given equal transmission through the female gamete, the relative male transmission frequencies can be calculated to be 0.61 and 0.39 for 1D and 1DL-1RS, respectively. The higher male transmission of a gamete bearing 1D is probably due to the greater vigour of pollen carrying a normal 1D over that bearing the translocation chromosome. A similar depression in male transmission of the complementary 1DS-1RL translocation from a translocation heterozygote was noted by Koebner and Shepherd (1985).

The three phenotypes recovered in the control population correspond to the two parental homozygotes and the heterozygote between them, and thus there was no evidence of recombination within the 1DS chromosome in the presence of chromosome 5B. However, in the nullisomic 5B families, 15 progeny with non-parental phenotypes were detected, representing putative recombinants.

The single seed scored as "- - -" lacked markers for both 1DS and 1RS and it was considered to be hypoploid rather than recombinant. Although aneuploid female gametes were found to function frequently in the test-cross experiment (Table 1), functional aneuploid male gametes are expected to be rare, so that few nullisomic progeny were expected in these selfed progeny.

It was planned to check the recombinant status of the remaining 14 seeds using progeny tests. However, only 12 of them could be germinated and progeny seeds were obtained by self-fertilisation and/or by pollination with Dit 1DL. These seeds were analysed by SDS-PAGE to elucidate the gametic constitution of the original putative recombinants. The observed protein phenotypes of progeny from crosses of 11 of these plants with Dit 1DL and from self fertilisation of 2 of them are shown in Table 4. The critical progeny are those with non-parental marker gene combinations

Table 4. Endosperm protein phenotypes and their frequency in two types of progeny from 12 of the putative recombinants isolated from 1D/1DL-1RS heterozygotes nullisomic for 5B

Recombinant plant no.		Endosperm protein phenotype					
	Tri-1	−	−	−	−	+	+
	Gli-D1	−	−	+	+	−	−
	Sec-1	+	−	+	−	−	+
(a) I-66		1		1			
I-93		7	2				7
II-83		3				3	
III-94		2	1			1	
III-95		3				3	1
IV-14		2	1			2	
VII-36		2	3			3	
VII-53		4				3	
VII-98		4				2	
I-45		4		2			
VI-59		3			2		
(b) III-1						2	4
I-66		2		3			

+ = protein present; − = protein absent (a) = Progeny from test-crosses to Dit 1DL; (b) = Progeny from self-fertilisation

and in particular dissociated *Tri-D1 Gli-D1* combinations, that is "−+" and "+−". These tests confirmed the recombinant status of all 12 plants. However, the presence of the Sec-1 protein in the small number of progeny analysed having dissociated *Tri-D1* and *Gli-D1* markers does not necessarily indicate that the *Sec-1* locus was also present on the recombinant chromosome. Dissociation of *Tri-D1* from *Gli-D1* could have resulted from pairing and recombination of 1DS with a homoeologous wheat chromosome arm rather than with the rye arm. In this case, the 1RS arm would not be altered, and the *Sec-1* locus would segregate independently from *Tri-D1* and *Gli-D1*. Additional progeny were analysed to determine this and it was confirmed that *Sec-1* is associated with a wheat marker in plants I-66 and I-93, whereas *Sec-1* segregated separately from *Gli-D1* in the progeny of I-45. From the data in Table 4 and these additional tests (including

tests for stem rust reaction) the distribution of marker loci on the recombinant chromosomes could be inferred, as shown in Table 5.

The 12 recombinants could be grouped into four different classes, depending on the combinations of the marker genes *Tri-D1*, *Gli-D1* and *Sec-1* present (Fig. 3). Two of these classes ("+−−" and "−+ +") appeared to be the same as those identified earlier among the test-cross progeny from 1D/1DL-1RS *ph1bph1b* plants (Table 2, Fig. 1). The two other classes of recombinants with "−+−" (plants I-45, VI-59) and "+−+" (plant I-93) classifications are apparently new combinations and represent separation of *Gli-D1* from *Tri-D1* in the first case, and association of *Sec-1* with *Tri-D1* in the second. Only two (I-66 and I-93) of these 12 additional recombinants appear to have come from homoeologous recombination between rye and wheat chromatin. The remainder probably resulted from recombination following pairing of 1DS with one of its wheat homoeologues.

A total of 528 of the progeny seeds derived from nullisomic 5B parents and 221 of the control progeny could be germinated and the seedlings were inoculated with stem rust. As in the test-cross experiment, resistance to the rust occurred only when the corresponding endosperm carried Sec-1. Thus in no case did 5B-nullisomy induce a break in the linkage between *Sec-1* and *SrR*.

1BL-1RS. The embryo halves of the four nullisomic 5B selections were planted in pots in the glasshouse, grown and allowed to self-fertilise. The progeny seeds were analysed by SDS-PAGE for the presence of Gli-B1 and Sec-1 proteins (Fig. 4). *Gli-B1*, the prolamin locus on chromosome arm 1BS codes for a group of three proteins (Gli-B1) of lesser mobility than Gli-D1 in cv. 'Chinese Spring'.

Table 5. Marker loci present on the recombinant chromosomes in 12 plants selected from progeny of 1D/1DL-1RS heterozygotes nullisomic for 5B, with respect to storage protein loci on chromosome arms 1DS and 1RS, and a rye locus conferring resistance to stem rust

Rec. type[a]	1DS loci		1RS loci	
	Tri-D1	*Gli-D1*	*Sec-1*	*SrR*
A	−	+	+	+
B	+	−	+	+
C	+	−	−	−
D	−	+	−	−

[a] Recombinant types A = I-66; B = I-93; C = II-83, III-1, III-94, III-95, IV-14, VII-36, VII-53, VII-98; D = I-45, VI-59
+ = locus present; − = locus absent

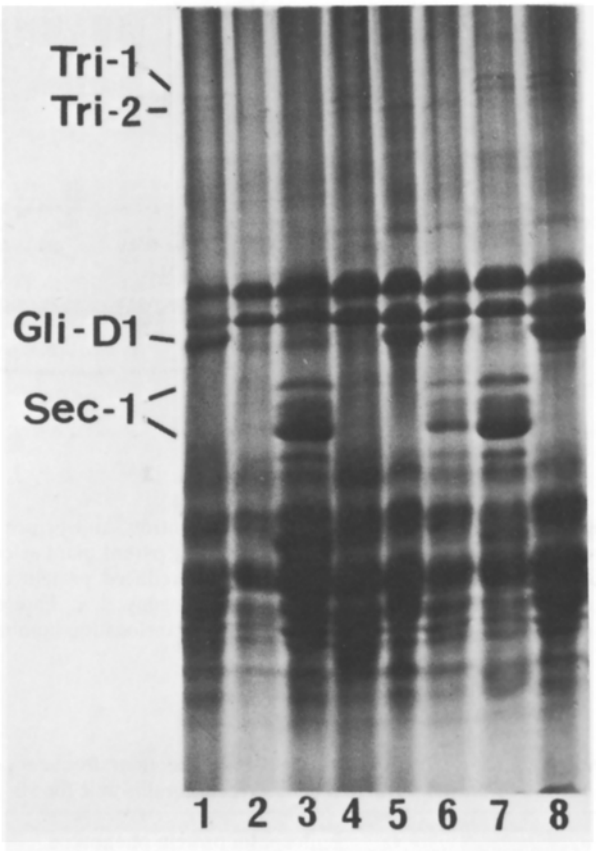

Fig. 3. Storage protein phenotypes of controls and the four different recombinant classes induced by chromosome 5B nullisomy. Patterns obtained by SDS-PAGE of unreduced protein extracts. Controls: *1, 8* 'Chinese Spring' euploid; *2* Dit 1DL; *3* Translocation 1DL-1RS. Recombinants: *4* Tri-1$^+$Gli-D1$^-$Sec-1$^-$ (e.g. II-83); *5* Tri-1$^-$Gli-D1$^+$Sec-1$^-$ (e.g. I-45); *6* Tri-1$^-$ Gli-D1$^+$Sec-1$^+$ (I-66); *7* Tri-1$^+$Gli-D1$^-$Sec-1$^+$ (I-93)

A total of 647 progeny were analysed and their SDS-PAGE phenotypes are listed in Table 6. The data exhibit significant heterogeneity ($\chi^2 = 19.34$, 6 d.f. $P < 0.01$) and thus cannot be pooled. The three major phenotypic classes were assumed to represent normal 1B homozygotes ("+−"), 1B/1BL-1RS heterozygotes ("+ +") and 1BL-1RS homozygotes ("−+"), while the single "−−" individual from 83-129-2 was thought to be a rare nullisomic progeny. Although pooling of the data is not warranted, it is evident that the segregation ratio among the three phenotypes departs from the expected 1:2:1, and since Singh (1985) has shown that transmission of the 1BL-1RS translocation chromosome in female gametes is normal, it is likely that the pollen bearing this translocation must suffer a competitive disadvantage.

In a search for allosyndetic recombination between the rye loci *SrR* and *Sec-1*, the 647 progeny were

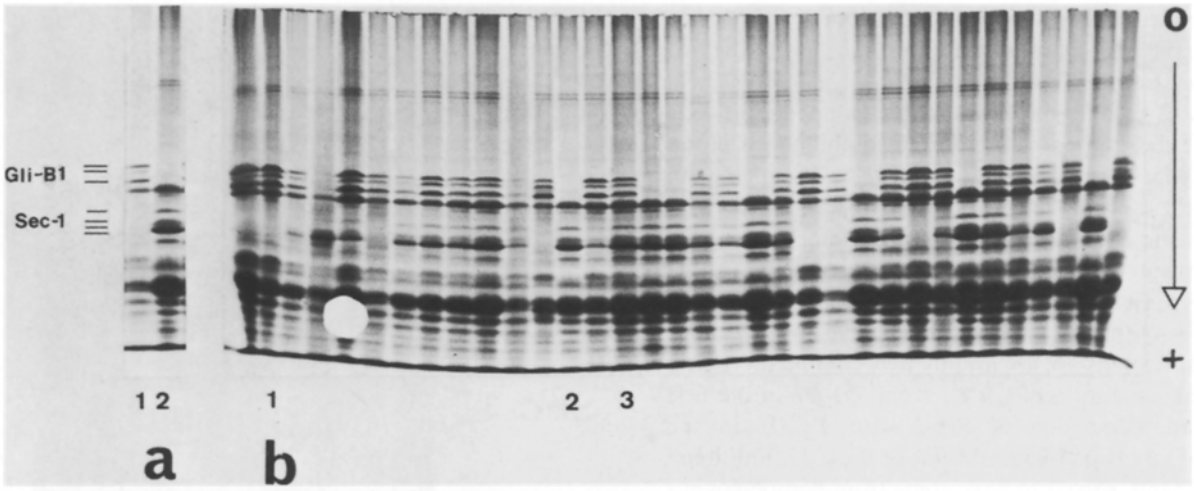

Fig. 4 a, b. Storage protein phenotypes of controls, and F_2 progeny from the cross (mono 5B × translocation 1BL-1RS) × N5BT5A, showing segregation for Sec-1 and Gli-B1. F_1 parent plant selected to be a 1B/1BL-1RS heterozygote, nullisomic for chromosome 5B. Patterns obtained by SDS-PAGE of unreduced protein extracts. **a** Controls: *1* 'Chinese Spring' euploid (Gli-B1$^+$Sec-1$^-$); *2* translocation 1BL-1RS (Gli-B1$^-$Sec-1$^+$). **b** Progeny: *1, 2, 3* are examples of the three major phenotypes recovered: *1* Gli-B1$^+$Sec-1$^-$ (normal 1B homozygote); *2* Gli-B1$^-$Sec-1$^+$ (translocation homozygote); *3* Gli-B1$^+$Sec-1$^+$ (translocation heterozygote)

Table 6. Endosperm protein phenotypes and their frequency in progeny from 1B/1BL-1RS heterozygotes, nullisomic for 5B

		Endosperm protein phenotype				
Family no.	Gli-B1 Sec-1	+ −	+ +	− +	− −	Total
84-128-1		54	128	52	0	234
84-129-1		8	9	5	0	22
84-129-2		69	82	19	1	171
84-130-1		58	116	46	0	220
Total		189	335	122	1	647

+ = protein present; − = protein absent

germinated and the seedlings inoculated with stem rust. All but four of the resulting 607 plants showed a complete association between the presence or absence of the Sec-1 bands and resistance or susceptibility, respectively, to stem rust. Two progeny seeds (C-89, D-69) lacked Sec-1 proteins, but gave seedlings which were resistant to stem rust, whereas two other progeny (F-39 and C-62) possessed rye proteins in their endosperm, but gave seedlings which were susceptible to stem rust. All four of these putative recombinants were progeny tested, and three (C-89, D-69, C-62) were confirmed to have recombinant phenotypes. However, all progeny from the stem rust susceptible plant F-39 lacked *Sec-1;* in this case either the parent had been wrongly classified for protein phenotype or the chromatin bearing the rye protein gene may have been present in the endosperm but not the embryo. Alternatively, it may have been lost during the growth of the plant, as has been observed for a telosome known to be present in the root tip meristem but absent in the meiocytes (Steinitz-Sears 1966).

Discussion

Recently it was demonstrated that allosyndetic recombination could be induced between the long arm of rye chromosome 1R and wheat chromosomes (Koebner and Shepherd 1985). The present work extends these findings to the agronomically important short arm of rye chromosome 1R. In particular, 1RS was recombined with a wheat chromosome three times in the 1DL-1RS experiments (82-180: Table 2; I-66, I-93: Table 5), and three times in the 1BL-1RS experiment.

The rate of allosyndetic recombination along chromosome arm 1RL was estimated to be 1.4% (Koebner and Shepherd 1985), and it is of interest to make a similar estimate with respect to 1RS. The gametic wheat-rye recombination frequency obtained in the *ph1bph1b* experiment can be calculated directly, as each test-cross gamete was analysed (1/394 = 0.3%). However, to obtain this frequency in the 1DL-1RS nullisomic 5B experiment it is necessary to estimate the transmission rates of a complete translocation chromosome, as recombinant gametes can only be detected when they unite with a normal translocation gamete. As the female transmission of the 1DL-1RS chromosome is 0.5 (Table 1), the male transmission of this chromosome can be estimated from the

data of Table 3 to be 0.39. If the recombinant chromosome suffers no selective disadvantage in either mega- or microsporogenesis, and recombination occurs with a probability P in either gamete, then the expected relative frequency of recombinant progeny is given by $(0.5+0.39)\,P$ and this can be equated to the observed relative frequency of $2/531=0.0038$, giving 0.4% as an estimate for P. In the 1BL-1RS experiment, recombinants could only be detected when a translocation chromosome was not transmitted; thus in this case the expected relative frequency of recombination is given by $(0.5+0.62)\,P$, which is equated to the observed relative frequency of $3/647=0.0046$, giving a similar estimate of 0.4% for P. These estimates for the rate of allosyndetic recombination are lower than that achieved for chromosome arm 1RL; however this was expected as the 1RL markers are known to span much of the genetic length of the arm, whereas those in in the 1RS experiments are less well spaced. This underlines the need for more genetic markers in order to increase the likelihood of detection of recombinants. In particular it would be more efficient to use markers specific to the rye arm, rather than relying on the indirect evidence of disruption of wheat linkage groups for the detection of wheat-rye recombinants. Additional markers are also useful to refine the analysis of recombinant lines, and they have been used to this effect as described in a companion paper (Koebner et al. 1986).

Recombination between *SrR* and *Sec-1* on chromosome arm 1RS was not obtained in either of the experiments involving the 1DL-1RS translocation, although three such recombinants were detected among 647 individuals in the case of 1BL-1RS. Linkage mapping of the 1DL-1RS chromosome has indicated that the likely gene order along the rye arm is: centromere – *Sec-1* – *SrR* – telomere, and that *Sec-1* and *SrR* are tightly linked (Singh 1985). Thus the probability of obtaining a homoeologous crossover between these two rye loci is likely to be low.

In one of the wheat-rye recombinant types, *Sec-1* has been transferred to the same chromosome as *Gli-D1* while *Tri-D1* has been lost, and in the other *Sec-1* has become associated with *Tri-D1* with the loss of *Gli-D1*. Previously it was assumed that *Gli-D1* and *Sec-1* are homoeoloci on the basis that they code for proteins having similar physical properties and, more strikingly, that their N-terminal amino-acid sequences show substantial homology (Shewry et al. 1984b). Data of Singh (1985), obtained from mapping 1R in a wheat background, show that *Sec-1* maps much closer to the 1R centromere than does *Gli-D1* to the 1D centromere. A similar lack of consistency in map distance between rye and wheat homoeoloci has been found for the *Glu-1* genes, where the rye locus is more tightly linked to its centromere than the wheat loci to their respective centromeres (Payne et al. 1982; Singh and Shepherd 1984). Thus the prolamin genes appear to be located at dissimilar positions genetically along their respective chromosome arms, and the evidence that they can be recombined on to a single chromosome arm is consistent with this conclusion. These data indicate that inferences concerning homoeologous relationships between genes should not be made on the basis of their map position alone.

In the present work two different approaches were used to generate the populations among which allosyndetic recombinants were sought. With the test cross procedure only the female gamete of the *ph1bph1b* individual was analysed, whereas in the nulli-5B F_2 populations both gametes were sampled. The main advantage in using F_2 populations is the avoidance of time-consuming emasculation and hand-pollination, as mentioned by Koebner and Shepherd (1985). Such populations are most efficient for detecting recombination when the characters to be scored are codominant, since then each individual progeny can be fully classified, and thereby provide twice as much information as a test cross individual (Mather 1936). However, when the characters are fully dominant, the F_2 procedure can only be used efficiently when the genes are closely linked in coupling. Even then recombinant gametes can only be detected when they unite with one of the two parental gametes, so that the efficiency is reduced to 1/2 of that where the characters are codominant. In this case the efficiencies of the test cross and the F_2 procedures are nearly equal (Mather 1936).

From an analysis of the interaction between *Ph1* and the pairing promoter gene(s) on the opposite arm of 5B, Riley and Chapman (1967) concluded that maximal homoeologous pairing would be achieved when *Ph1* was removed or suppressed while the 5BS pairing promotion activity was retained. It follows that the frequency of allosyndetic recombination should be higher with the *ph1b* mutant rather than with nullisomy for 5B. In the present study, the overall rate of allosyndetic recombination in the interval *Tri-D1* to *Gli-D1* among the test-cross progeny from the *ph1b* homozygote was 1.0% (4 out of 394). The equivalent recombination rate obtained in the nullisomic 5B 1DL-1RS experiment was calculated assuming that the female and male transmissions of the 1DL-1RS chromosome are 0.5 and 0.39, respectively. This gives an expected relative frequency of recombination of $(0.5+0.39)\,P$ which, when equated to the observed relative frequency of 14/531 ($=0.026$), gives an estimate for P of 2.9%, or almost three times that achieved in the *ph1bph1b* test-cross experiment. Thus it appears that nullisomy for 5B significantly increases the rate of allosyndetic recombination over that achieved by *ph1b*, but these data should be treated with caution, as the experimental material was grown at different times of the year, and different methods of obtaining the progeny were employed.

The use of 5B nullisomy has the further advantage that it is relatively easy to identify those critical individuals which lack chromosome 5B, compared to the difficulty of identifying homozygosity for *ph1b* by

cytological screening and test crosses to *Ae. variabilis* (Koebner and Shepherd 1985). The major disadvantage of the nullisomic 5B method lies in the poor fertility of some of these plants; however the problem of low seed yield can be overcome by simply selecting a greater number of nullisomic 5B parents.

The short arms of homoeologous group 1 chromosomes of wheat carry genes coding for the non-aggregating gliadin storage proteins (Shepherd 1968; Wrigley and Shepherd 1973), for the aggregating low-molecular-weight (LMW) glutenin subunits (Jackson et al. 1983; Payne et al. 1984; Singh and Shepherd 1985) now given the gene designation *Glu3* (Singh 1985) and for a new class of aggregating proteins given the provisional designation 'triplet' (*Tri*) (Singh and Shepherd 1985). Both the quantity and quality of protein in the endosperm have a major influence on the breadmaking quality of dough (Finney and Barmore 1948; Bushuk et al. 1969). An association between four quality attributes and gliadin phenotype was observed by Wrigley et al. (1982a), and the presence of certain gliadin bands was found to be closely correlated with particle size index and dough strength by Wrigley et al. (1982b). Although these correlations were not claimed to be causal, the involvement of many protein species in the gliadin phenotypes correlated with higher quality suggests that this is at least possible. A strong correlation between dough strength and the presence of a single gliadin band on electrophoregrams of total protein extracts of durum wheat has been observed by Damideaux et al. (1978). The *Tri-D1* locus and the *Gli-D1/Glu-D3* complex are genetically separate on chromosome arm 1DS, and these loci code for proteins which are expected to differ in their effect on dough quality. If the rye arm is neutral in its effect on quality, that is, the poor quality of the wheat-rye translocations is due to loss of wheat chromatin rather than to gain of rye, then it can be expected that the lines with phenotype Tri-1$^-$Gli-D1$^+$Sec-1$^+$D1$^-$ will differ in quality from those with phenotype Tri-1$^+$Gli-Sec-1$^+$. It will be of interest to determine whether the quality problem associated with the 1DL-1RS translocation has been overcome, at least partially, in one or other of these two recombinant types. The influence of the 1DL-1RS translocation on flour quality cannot be reliably measured in a 'Chinese Spring' background, as this cultivar itself produces a dough which is very weak (Shepherd and Singh 1984). The wheat-rye recombinants therefore are being backcrossed to cv. 'Gabo', chosen as the recurrent parent because it has been the breadmaking quality standard for Australian wheats. Lines homozygous for the recombinant chromosome, and sister lines homozygous for normal 1D and for the translocation 1DL-1RS will be isolated, and these lines will permit an assessment of the effect on dough quality of these allosyndetic recombinants.

The wheat-rye recombinants can be used further to test the suggestion of Zeller et al. (1982) that the gene product of *Sec-1* may be the cause of the sticky dough problem in 1BL-1RS lines. Since the Tri-1$^+$Gli-D1$^-$Sec-1$^+$ SrR$^+$ and Tri-1$^-$Gli-D1$^+$Sec-1$^+$SrR$^+$ chromosomes share some rye chromatin, the rye segment can now be further shortened by homologous recombination according to the scheme suggested by Sears (1981). A genotype possessing all four loci should be obtainable; this chromosome will possess all the known 1DS seed storage protein genes, and may therefore be free of the dough quality problem associated with 1DL-1RS translocation if the cause of the defect is loss of wheat genes rather than the acquisition of deleterious effects due to rye genes. If there are deleterious genes affecting dough quality on the full rye arm, it is possible that one or more of the recombinant lines will have lost these genes and will not produce sticky dough.

The recombinant 1DS chromosomes produced in this study may provide further information on the genetic control of the dough quality problem associated with the 1DL-1RS translocation. Lines possessing the Tri-1$^+$Gli-D1$^-$Glu-D3$^-$ recombinant chromosome in a suitable wheat background will demonstrate the effect on flour quality of the absence of this particular gliadin and low molecular weight glutelin subunit, and the lines with the Tri-1$^-$Gli-D1$^+$Glu-D3$^+$ recombinant chromosome will similarly provide information on the effect of the 'triplet' proteins Tri-1 and Tri-2 on quality.

The two presumptive recombinants arising from 1BL-1RS, in which *SrR* has been separated from *Sec-1* are also being backcrossed to cv. 'Gabo' to assess whether the novel translocations have improved the quality characteristics of the original translocation stock. If these lines produce flour of an adequate strength, then the rye gene for stem rust will be available for immediate inclusion into conventional wheat breeding programmes. An intercross between the two recombinant types (Sec-1$^-$Sr$^+$ and Sec-1$^+$Sr$^-$) will enable the rye segment to be further shortened if the recombinant chromosomes carry any common rye chromatin, but genotypes deriving from homologous crossing-over within the common rye segment will not carry *SrR*, and thus will not be of direct use in wheat breeding at present.

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