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

2. Characterisation of recombinants

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Summary. Sixteen allosyndetic recombinants involving chromosome arms 1DS and 1RS derived from the wheat-rye translocation 1DL-1RS, and one 1RS recombinant derived from 1BL-1RS were characterised by cytological, biochemical and molecular methods. Analysis of isozyme markers showed that recombination had involved different breakpoints along the 1DS arm. The gene order *Tri-D1 – Per-D1 – Gpi-D1 – Gli-D1* was established on chromosome 1DS, and this is consistent with the gene order known to occur on 1BS. The use of three molecular probes for loci on 1RS confirmed one of the recombinants obtained from the 1BL-1RS translocation, and two of the three recombinants obtained from the 1DL-1RS translocation. These two recombinant chromosomes appear to have approximately reciprocal structures and may prove of direct benefit to wheat breeders as they retain the stem rust resistance of the parental translocation but have recovered some of the 1D encoded proteins which are absent in 1DL-1RS lines and which are thought to affect dough quality. A 5S RNA site was discovered on chromosome arm 1DS, probably reflecting the homoeologous relationship of this chromosome with 1BS. Segregational analysis indicated that some of the homoeologous wheat-wheat exchanges probably involved more than a single allosyndetic event.

Key words: Allosyndetic recombination – Wheat-rye exchanges – Isozyme markers – Molecular markers

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Introduction

Several wheat-rye translocations are now known involving 1RS of rye and wheat chromosomes 1BL (Mettin et al. 1973; Zeller 1973; Shepherd, unpubl.), 1DL (Shepherd 1973) and 1AL (Zeller and Fuchs 1983). The interest in this material stems from the occurrence of several genes on the rye arm controlling resistance to foliar diseases (as listed in Koebner and Shepherd 1986a) and from the positive effect on yield associated with breeding lines carrying, in particular, the 1BL-1RS translocation (Rajaram et al. 1983). The presence of these translocations is, however, commonly associated with sticky or weak dough, and thus these lines are generally unsuitable for breadmaking (Zeller et al. 1982; D. J. Martin, personal communication). This quality defect has been observed in many wheat backgrounds (Shepherd and Singh 1984), and the induction of allosyndetic recombination between the rye chromosome arm and its wheat homoeologues was seen as a possible means of overcoming this problem, either through removal of deleterious genes from the rye segment and/or by reincorporation of wheat genes of importance to breadmaking quality which are absent in these supposed centric fusion translocation lines (Koebner and Shepherd 1986a).

Koebner and Shepherd (1986a) have utilised both the *ph1b* mutant (Sears 1977) and nullisomy for chromosome 5B in order to induce a number of allosyndetic recombinants in translocation heterozygotes, i.e. those plants having the chromosome combinations normal 1D/translocation 1DL-1RS or normal 1B/translocation 1BL-1RS. Progeny from these plants were screened for recombination between markers on 1DS and 1RS in the 1DL-1RS heterozygotes, and on 1RS alone in the 1BL-1RS heterozygotes. In the former case, 13 wheat-

wheat and 3 wheat-rye recombinants were isolated, while in the latter case 3 wheat-rye recombinants were detected. At the time of their isolation little was known of the structure of these recombinant chromosomes, that is, which wheat chromosomes had participated in the recombination event and how much rye chromatin was present in the wheat-rye recombinants. In this paper, a variety of cytological, biochemical and molecular techniques have been employed in order to further characterise these allosyndetic recombinants.

Materials and methods

1 Plant materials

The same wheat stocks were used as listed in Koebner and Shepherd (1986a), with the addition of ditelosomic 1BL (Dit 1BL) in cv. 'Chinese Spring' (Sears and Sears 1978). The allosyndetic recombinants analysed comprised the 16 derived from 1DL-1RS (plant numbers 82-177, -178, -179, -180 obtained in the *ph1b* test-cross progeny, and 1-45, -66, -93; II-83, III-1, -94, -95; IV-14; VI-59; VII-36, -53, -98 obtained in progeny from plants nullisomic for 5B) and one of the three wheat-rye recombinants obtained from 1BL-1RS (plant C-62). The endosperm protein phenotypes and stem rust reactions of these plants have been described in a companion paper (Koebner and Shepherd 1986a) and are included in Table 1.

2 Marker loci used to characterise the recombinants

Chromosome arm 1RS possesses a gene for a prolamin storage protein *Sec-1* (Shepherd and Jennings 1971; Shewry et al. 1984) and a gene for resistance to stem rust (*SrR*); chromosomes 1R of *Secale montanum* and *S. cereale* cv. 'King II' possess a structural gene for the isozyme glucose phosphate isomerase (Gpi) (Chojecki and Gale 1982), and analysis of lines carrying only 1RS of *S. cereale* cv. 'Imperial' have shown that the locus is present on the short arm of the rye chromosome (Koebner, unpubl.). Three molecular markers – rDNA, 5S RNA (Appels et al. 1978) and the 350-family telomeric heterochromatin sequence (Appels and Moran 1984) – have been located in the satellite region of 1RS.

Chromosome arm 1DS carries genes for the ω-gliadin storage protein Gli-D1 (Shepherd 1968; Payne et al. 1982), the LMW glutelin subunit Glu-D3 and the triplet proteins Tri-1 and Tri-2 (Payne et al. 1984; Singh and Shepherd 1985); structural genes for the isozymes Gpi (Chojecki and Gale 1982) and leaf peroxidase (Per) (Ainsworth et al. 1984); and a 5S DNA site described in the present paper.

Chromosome arm 1BS carries the ω-gliadin gene *Gli-B1* (Shepherd 1968; Payne et al. 1982), and loci which cross-hybridise to the rye molecular probes for Nor-R1 (Appels and Dvorak 1982) and 5S RNA (Appels et al. 1980).

3 Electrophoretic separation of storage proteins and isozymes

SDS-PAGE of unreduced endosperm protein extracts followed the methods of Singh and Shepherd (1985). The isozymes of leaf peroxidase were visualised by flatbed isoelectric focusing (IEF) as described by Ainsworth et al. (1984). IEF was also employed to analyse glucose phosphate isomerase isozymes. The IEF procedure is described elsewhere (Koebner and Shepherd 1986b); however in order to separate the isozymes controlled by *Gpi-R1* it was necessary to flatten the pH gradient around pH 7 by the addition of 25 mg/ml β-alanine to the gel mixture (Righetti 1983). Sample extraction followed Chojecki and Gale (1982), except that only 50 µl of water was added to the ground half of a mature dry seed.

4 DNA extraction, restriction and hybridisation

Two tillers from 1–2 month old plants were ground in liquid N_2 with the aid of acid-washed sand (BDH) to form a fine powder. The powder was transferred to 5 ml of a solution containing 3.8 ml 0.1 M NaCl, 0.2 M EDTA, 0.05 M Tris-HCl pH 7.5, 0.6 ml 5% SDS and 0.6 ml proteinase K (0.5 mg/ml), and incubated at 37 °C for 1–2 h (Appels and Moran 1984, as modified by C. E. May and R. Appels (unpubl.)). Sodium perchlorate (1 g) was then added to the homogenate which was vortexed and centrifuged at 8000 rpm for 5 min to pellet the debris. The supernatant was mixed gently with 9 ml ethanol saturated with sodium perchlorate (Appels and Moran 1984) and the DNA scooped out with a spatula. The DNA was then resuspended in 0.6 ml TE (0.01 M Tris-HCl pH 8.5, 0.001 M EDTA) and extracted with 0.25 ml chloroform plus 0.25 ml phenol; to completely remove the green coloration it was found necessary to extract samples overnight at 4 °C. The DNA was finally precipitated with 1 ml ethanol containing 1

Table 1. Phenotype of allosyndetic recombinants from 1DL-1RS heterozygotes for biochemical, molecular and physiological markers

Recombinant plant type	Tri-1	Per-D1	Gpi-D1a	Gli-D1	Sec-1	Gpi-R1	Nor-R1	5S DNA	Het	SrR
Wheat-wheat:										
A	+	+	+	–	–	–				–
B	+	+	–	–	–	–				–
C	–	–	+	+	–	–				–
Wheat-rye:										
D	–	–	–	+	+	+	+	+	–	+
E	+	+	–	–	+	+	–	+	+	+
F	–	–	+	+	+	+?				+

A: 82-177, -179; II-83; III-1, -94, -95; IV-14; VII-36, -53, -98. B: 82-178. C: I-45, VI-59. D:82-180. E: I-93. F: I-66

+ = marker present; – = marker absent

volume of 2 M NaAc pH 5.0, washed with 70% ethanol and resuspended in 0.8 ml of TE to give an approximate concentration of 1 mg/ml.

The DNA samples were digested with the restriction endonuclease TaqI and then electrophoresed on 1.5% or 2% agarose gels, following standard procedures. Transfer of DNA fragments from the gel to GENE-SCREEN (New England Nuclear) followed the manufacturer's instructions. Filters from the transfer were baked at 80 °C under vacuum for 2–3 h, soaked in PH buffer (3×SSC, 50% formamide [Fluka], 0.1% SDS, 0.01% bovine serum albumin, 0.01% ficoll, 0.01% polyvinylpyrrolidone, 0.01 M Tris-HCl pH 8.5, 0.001 M EDTA) for approximately 2 h, and bathed in PH buffer containing the radioactive probe at a concentration of 0.02–0.04 μg/ml for 12 h at 37 °C. The plasmids containing the probe sequences were rendered radioactive by nick-translation (nick translation kit, BRESA, South Australia). Following hybridisation, the filters were washed in a solution of 2×SSC and 0.1% SDS (initially at 65 °C) for 1–2 h. The washing solution was usually changed three times during this period. Excess moisture was then removed from the filters, which were wrapped in Clingwrap for exposure to flashed X-ray film at –80 °C with the aid of an intensifying screen.

Results

1 Characterisation of parental and recombinant genotypes using biochemical and molecular markers

1.1 Parental phenotypes

(a) Leaf peroxidase. The genetic control of certain wheat peroxidase isozymes separated by isoelectric focusing (bands labelled 1–8) has been elucidated by Ainsworth et al. (1984). In the absence of chromosome arm 1DS, bands 6 and 7 are missing. However, in the present work it was found that band 6 is not a reliable marker for *Per-D1*, and examination of a number of the figures given by Ainsworth et al. (1984) supports this conclusion. Thus only band 7 was used as an indicator for the presence of this locus (Fig. 1).

(b) Glucose phosphate isomerase. In cv. 'Chinese Spring', three bands in the central portion of the gel are absent when chromosome 1DS is deleted (bands W1, W2, W3). When this chromosome arm is replaced by 1RS, as in the translocation line 1DL-1RS, at least one new band (R1) is produced (Fig. 2).

(c) Nor-R1. The DNA sequence in pScR4-T1 assays the Nor-R1 locus. When this sequence is hybridised to TaqI digests of total plant DNA of cv. 'Chinese Spring', three bands are observed on Southern blots (3.1 kb, 2.8 kb, 2.7 kb: Fig. 3). Genotypes possessing the rye locus Nor-R1 give an additional heavy band (2.4 kb) in these gels.

(d) 5S RNA. The DNA sequence in pSc 5S-T7 assays the 5S RNA locus. From in situ studies a major location of 5S RNA is known to be interstitial between *Nor-1* and the telomere on both 1RS and 1BS (Appels et al. 1980). When this sequence is hybridised to TaqI digests of total plant DNA of cv. 'Chinese Spring', two bands are observed on Southern blots (0.27 kb, 0.22 kb: Fig. 4). The 0.22 kb fragment is most likely derived

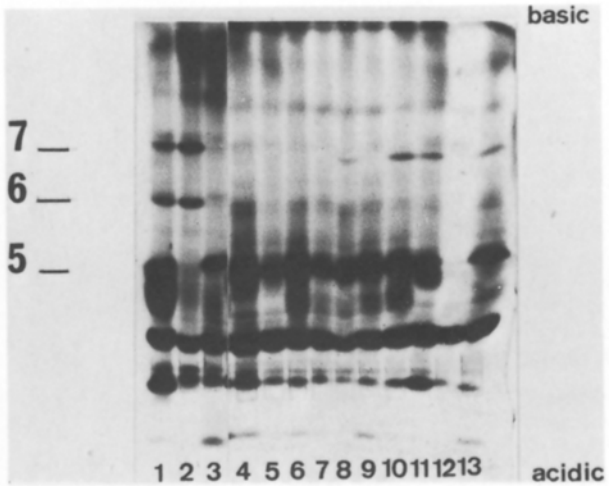

Fig. 1. Leaf peroxidase zymogram of wheat, wheat-rye stocks and allosyndetic recombinant types. The phenotype with respect to bands 5, 6 and 7 (Ainsworth et al. 1984) is given in brackets. Parents and 'Chinese Spring' (CS) aneuploids: *1, 13* CS (bands 5, 6, 7); *2* Dit 1BL (6, 7); *3* Dit 1DL (5); *4, 6* 1DL-1RS (5, 6?); *12* 1BL-1RS/1DL-1RS (null). Recombinants: *5* 82-180 (5, 6?); *7* I-66 (5, 6?); *8* I-93 (5, 6?, 7); *9* VI-59 (5, 6?); *10* 82-177 (5, 6?, 7); *11* 82-179 (5, 6?, 7)

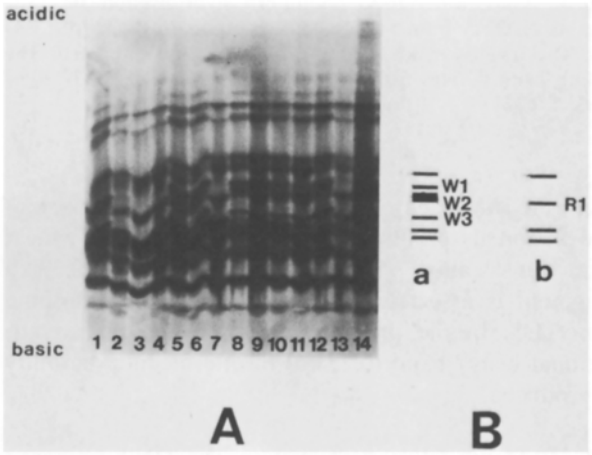

Fig. 2 A, B. Glucose phosphate isomerase zymogram of wheat, wheat-rye stocks and allosyndetic recombinant types. **A** Parents: *1, 6, 12* 'Chinese Spring'; *2, 4, 13* 1DL-1RS. Recombinants: *3* 82-180; *5* I-66; *7* I-93; *8* VI-59; *9* VII-36; *10* VII-53; *11* VII-98; *14* IV-14. **B** Interpretation of the banding pattern in the the mid section of the gel (around pH 7) shown in **A** of (a) 'Chinese Spring', (b) 1DL-1RS. Major wheat bands denoted by W1, W2, W3; major rye band by R1. Bands W1, W2, W3 controlled by *Gpi-D1.* Band R1 controlled by *Gpi-R1*

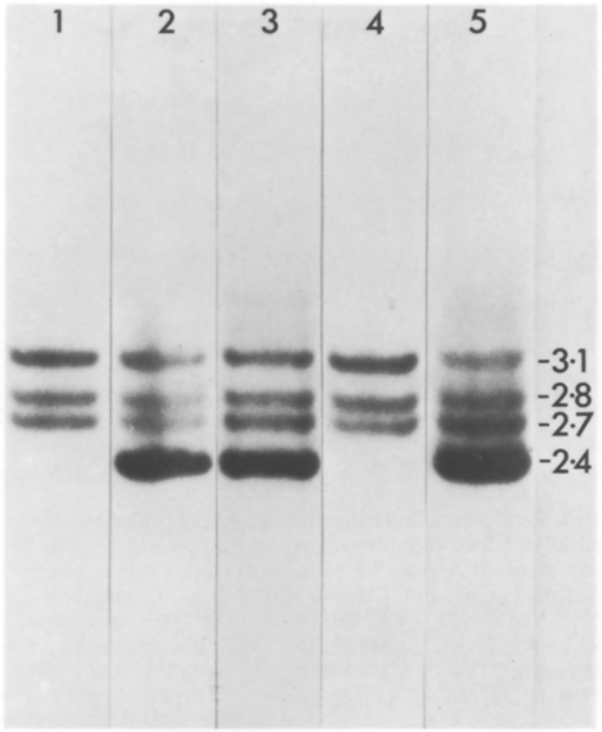

Fig. 3. Assay for *Nor-R1*. Southern hybridisation of TaqI digests of DNA from parents and recombinants with pSc R4-T1. The lengths in kb of the TaqI bands are indicated. The 2.4 kb band derives from 1RS. *1* CS; *2* 1DL-1RS; *3* 82-180; *4* I-93; *5:* C-62

from the site on chromosome arm 1BS. Comparison of the fragments produced by digestion of normal wheat and translocation 1DL-1RS shows that the 0.27 kb fragment is associated with the missing chromosome arm 1DS. In the presence of the 1RS locus an additional heavy band (0.25 kb) of intermediate mobility is produced.

(e) 350-family telomeric sequence. The DNA sequence in pSc het 1 assays the rye telomeric heterochromatin. There is no hybridisation between this sequence and TaqI digests of total plant DNA from cv. 'Chinese Spring', but in the presence of the 1RS telomere, a single major band (0.38 kb) is produced on the Southern blot (Fig. 5); a minor band of 0.76 kb is often seen as a result of partial digestion by TaqI.

1.2 Recombinant phenotypes

1DL-1RS. Analysis of leaf peroxidase phenotypes of progeny of the recombinants indicated that the association between *Tri-D1* and *Per-D1* was always maintained, that is, all Tri-1$^+$ plants were also Per-D1$^+$, and vice versa, indicating a relatively close linkage between these two loci. Ainsworth et al. (1984) reported that a specific peroxidase isozyme is associated with chromosome 1RS in both cv. 'King II' and in the 1BL-1RS translocations Veery "S" and Hahn "S". However, no rye isozyme was detected on our gels even in the double translocation line 1BL-1RS/1DL-1RS which has four doses of the rye arm and lacks any enzyme activity associated with 1BS and 1DS (Fig. 1, track 12). Recently, Wehling et al. (1985) have reported that a structural gene for leaf peroxidase in 'Imperial' rye is present on chromosome 2R, and this could explain our failure to detect a band associated with 1RS.

The glucose phosphate isomerase phenotypes of the recombinants showed that of the 12 Tri-1$^+$ lines, only two (82-178 and I-93, Table 1) did not carry *Gpi-D1 a* (nomenclature of Chojecki and Gale 1982); while of the four Tri-1$^-$ lines, two retained this locus and two lacked it (Table 1). The *Gpi-R1* band could not be detected in the presence of *Gpi-D1 a,* but it was clearly present in the two wheat-rye recombinants 82-180 and I-93 (Fig. 2).

Only those recombinants known to carry rye markers (*Sec-1, SrR* and *Gpi-R1*) were analysed further using molecular markers specific for rye chromatin. Of

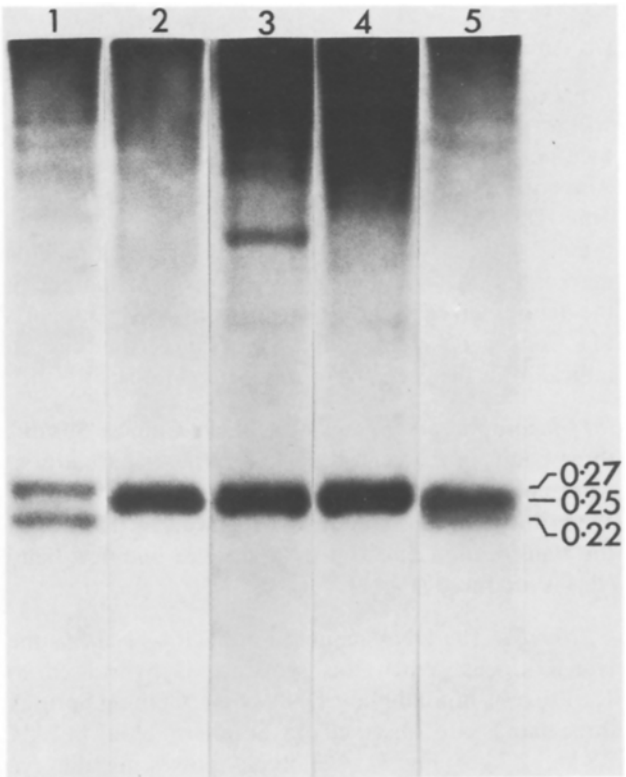

Fig. 4. Assay for *5S RNA*. Southern hybridisation of TaqI digests of DNA from parents and recombinants with pSc 5S-T7. The lengths in kb of the TaqI bands are indicated. The 0.25 kb band derives from 1RS. *1* CS; *2* 1DL-1RS; *3* 82-180; *4* I-93; *5* C-62

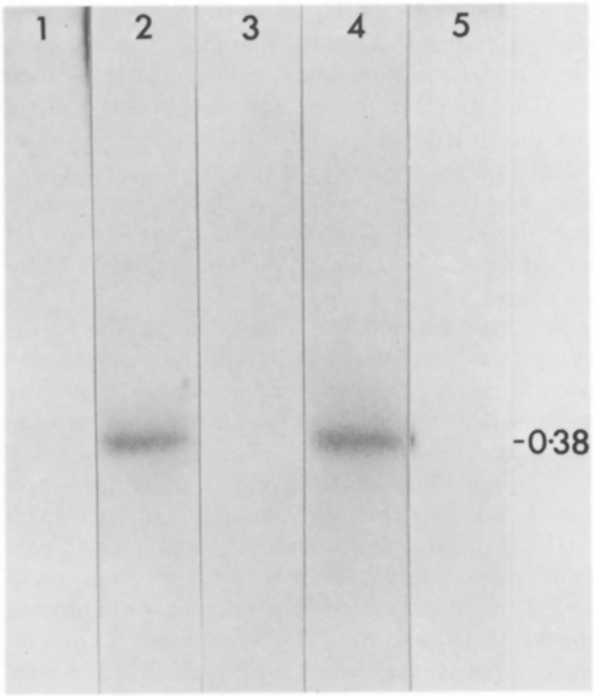

Fig. 5. Assay for 350-family telomeric heterochromatin. Southern hybridisation of TaqI digests of DNA from parents and recombinants with pSc het 1. The length in kb of the TaqI band characteristic of rye terminal heterochromatin is shown. *1* CS; *2* 1DL-1RS; *3* 82-180; *4* I-93; *5* C-62.

the three wheat-rye recombinants derived from 1DL-1RS only two (82-180, I-93) were studied, as the remaining putative recombinant (I-66) was not available in a homozygous condition. The three molecular probes used showed that 82-180 possessed *Nor-R1* and rye *5S RNA*, but lacked the telomeric heterochromatin; while, in contrast, I-93 retained the rye *5S RNA* locus and the heterochromatin but lacked *Nor-R1* (Figs. 5, 6, 7; Table 1).

1BL-1RS. Three putative wheat-rye recombinants were produced by observing a disruption of the association between *SrR* and *Sec-1* in populations derived from plants nullisomic for chromosome 5B (Koebner and Shepherd 1986a). Plant C-62 (Sec-1$^+$Sr$^-$) is the only one characterised using molecular probes thus far. Sec-1$^+$ progeny of plant C-62 retained both *Nor-R1* and the rye *5S RNA* locus, but lacked the rye heterochromatin, thereby establishing the recombinant nature of this plant. The presence of both *Nor-R1* and *Nor-B1* does not necessarily indicate an intra-locus recombination event, as the plant probably carries an intact chromosome 1B, as suggested by the continuing presence of *Gli-B1* in the progeny analysed. Analysis of the nature of this recombinant and the location of the breakpoint along 1RS will require the introduction of the C-62 chromosome into a background lacking 1BS.

2 Structure of the four recombinant chromosomes in plants 82-177, -178, -179 and -180

The recombinant chromosomes in the three plants 82-177, -178 and -179 (selected among progeny of a 1D/1DL-1RS heterozygote, homozygous for *ph1b* on the basis of a disruption of the *Tri-D1–Gli-D1* linkage on chromosome 1DS) apparently do not possess rye chromatin, as there was no evidence of any chromosome pairing with a normal chromosome 1R in hybrids with substitution (1D) 1R (Koebner and Shepherd 1986a). To obtain further information on the relationship between these three supposed wheat-wheat recombinants and the wheat-rye recombinant present in plant 82-180, the three plants 82-177, -178 and -179 were each crossed to plant 82-180. F_1 progeny possessing both recombinant chromosomes (SDS-PAGE phenotype Tri-1$^+$Gli-D1$^+$Sec-1$^+$) were selected, and these were test-crossed to Dit 1DL. The test-cross progeny were analysed by SDS-PAGE and seven different phenotypes were recovered as shown in Table 2.

The test-cross progeny from the combination 82-178 × 82-180 did not show any recombination between *Gli-D1* and *Sec-1* but these two loci recombined freely with *Tri-D1* leading to a 1 : 1 : 1 : 1 distribution among the four phenotypic classes. It is concluded that *Tri-D1*

Table 2. Endosperm protein phenotypes and their frequency among the progeny from the three test-crosses (82-177 × 82-180), (-178 × -180) and (-179 × -180) × Dit 1DL

Test-cross combination		Endosperm protein phenotype							
	Tri-1	+	–	+	–	–	+	+	
	Gli-D1	–	+	+	–	–	–	+	
	Sec-1	–	+	+	–	+	+	–	Total
(178 × 180) × Dit 1DL		27	31	26	25	0	0	0	109
(177 × 180) × Dit 1DL		33	28	18	15	3	1	0	98
(179 × 180) × Dit 1DL		47	61	2	0	9	0	5	124

+ = presence of protein; – = absence of protein

in 82-178 and *Gli-D1, Sec-1* in 82-180 are present on different chromosomes, which allows them to recombine freely. Furthermore, the short arm of the recombinant chromosome in 82-178 must not contain any chromatin homologous with that in 82-180 in the interval *Sec-1* to *Gli-D1*, because of the complete linkage observed between these two loci.

The two simplest models of chromosome structure consistent with this evidence are shown in Fig. 6 (a) and (b). In model (a) it is assumed that *Tri-D1* was separated from *Gli-D1* in 82-178 by recombination between chromosome arm 1DS and a wheat homoeologue. The more complex structure of 82-180 is assumed to have arisen following two episodes of homoeologous recombination in a trivalent involving 1D, 1DL-1RS and another homoeologue. In model (b) the complexity is assigned to the recombinant chromosome in 82-178 where a segment of 1D is shown to be located interstitially in another wheat homoeologue. Such a chromosome structure would require two homoeologous crossovers in a bivalent involving 1D and another homoeologue, or in a trivalent involving 1D and a pair of homoeologues or two different chromosomes. The 82-180 chromosome is assumed to have arisen from a single homoeologous crossover between 1RS and 1DS. Diagnostic crosses between the two recombinant lines and the homoeologous group 1 telocentric stocks are required to resolve which of these possibilities may be correct.

The phenotypic frequencies of the test-cross progeny from the 82-177×82–180 combination resemble those from the 82-178×82-180 combination, except for four individuals with the additional phenotypes

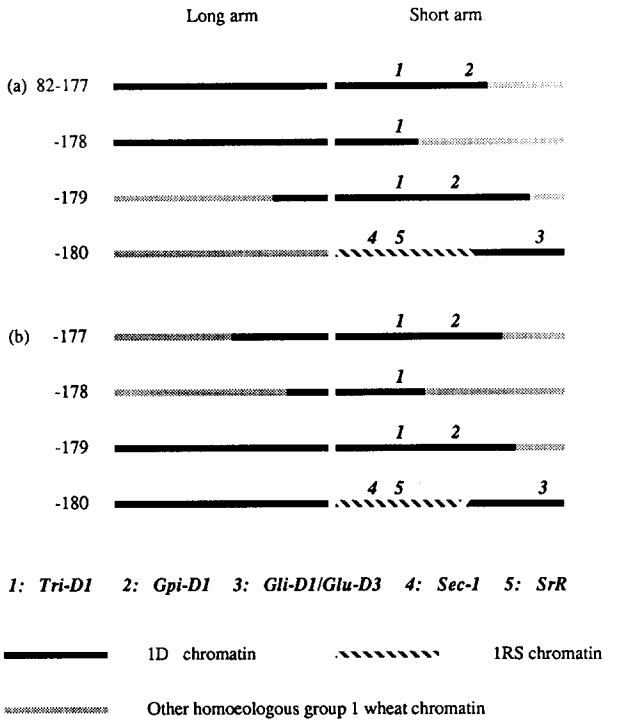

Fig. 6. Alternative models for the recombinant chromosomes present in plants 82-177, -178, -179 and -180. Note proposed common segment of 1DS chromatin in 82-179 and -180

"+−+" and "−−+" (Table 2). The results from progeny tests of these plants suggested that they probably arose from a mistake in pollination, with pollen being used from 1DL-1RS plants ("−−+") growing in pots adjacent to the proper test-cross parent Dit 1DL ("−−−"). Thus it is likely that the recombinant chromosomes present in plants 82-177 and 82-178 have a similar structure, except that the 82-177 chromosome carries the *Gpi-D1* gene whereas the 82-178 chromosome does not (Table 1).

The test-cross progeny from the 82-179×82-180 combination gave a markedly different segregation pattern from the other two combinations, with most of the progeny falling into one or other of the parental phenotypic classes (Table 2). The few "−−+" and "++−" individuals were thought to be cross-over products following chiasma formation in a short length of 1DS chromatin common to the two recombinant chromosomes (Fig. 6). The two "+++" individuals (Table 2) were progeny tested and found to possess both the 82-180 and the 82-179 recombinant chromosomes – that is, they were hyperploid. These results suggest that the 82-179 recombinant chromosome arose following homoeologous synapsis between 1DS and the short arm of another group 1 wheat chromosome.

In an attempt to find which group 1 wheat chromosomes were now associated with *Tri-D1* in 82-177 and 82-178, both lines were crossed and then backcrossed to the double translocation line 1DL-1RS/1BL-1RS. The recurrent parent lacks both the short arms of 1B and 1D and thus, in progeny from the first cross, both *Tri-D1* and *Gli-B1* will be present in only one dose, so that in the backcross they should always segregate together if they are both present on the same chromosome arm. Thus if *Tri-D1* had been recombined onto 1BS, the Tri-1 and Gli-B1 bands would be either both present or both absent in the backcross progeny. However, these bands segregated independently in both cases (Fig. 7). Since 1BS appears not to be involved, and since *ph1b* homozygosity is expected to induce homoeologous translocations, it is thought likely that plants 82-177 and 82-178 contain 1AS-1DS exchanges.

Discussion

The primary aim of this programme to induce allosyndetic recombination between rye chromosome 1RS and its wheat homoeologues was to shorten the length of rye chromatin present in the supposed centric fusion translocations 1DL-1RS and 1BL-1RS, where a whole rye arm is represented. This was undertaken in order to attempt to alleviate the dough quality defect associated with these translocations, so that the desirable alien

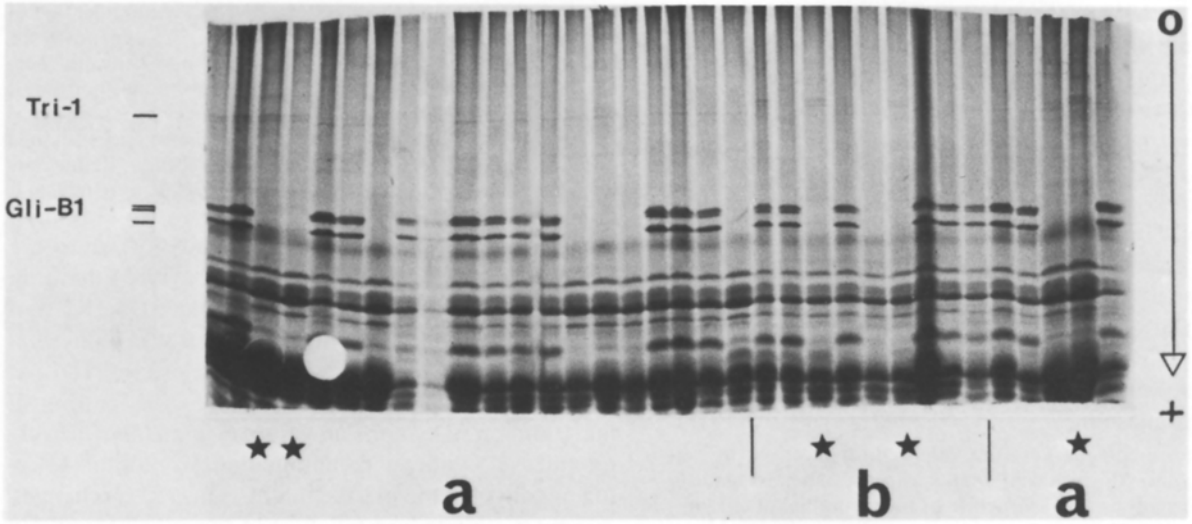

Fig. 7. Endosperm storage protein of BC1 progeny from the two crosses recombinant 82-177 and 82-178 × (double translocation 1BL-1RS/1DL-1RS)². Patterns obtained by SDS-PAGE of unreduced extracts. *Asterisks* mark progeny from (**a**) 82-178 and (**b**) 82-177 with the diagnostic phenotype Tri-1⁺Gli-B1⁻

disease resistance could be exploited in breadmaking wheats. Although it is still unclear whether the quality defect of the wheat-rye translocations is a direct effect of the presence of rye chromatin or rather an indirect effect resulting from the absence of critical wheat genes, the most useful recombinants would be expected to be those individuals resistant to stem rust which contain the shortest possible segment of rye chromatin. An approach for determining the amount of alien chromatin present in such lines is by analysis of genetic markers, and this requires as many chromosomal markers as possible. Thus lines with the most wheat markers and the fewest rye markers in addition to the target *SrR* gene would be predicted to have the greatest chance of being free of the quality problem associated with the 1DL-1RS and 1BL-1RS translocations. Thus it should be possible to choose between a number of recombinants, where these are available.

The allocation of the ten loci scored in the present work to the recombinant chromosomes present in plants 82-180 and I-93 suggests that they are approximately reciprocal types as shown in the physical models of these chromosomes (Fig. 8). Of particular note is the proposed common segment of rye chromatin in the two recombinants, so that, when these two recombinants are intercrossed, it should be possible to use homologous crossing-over to shorten the rye chromatin even further, as outlined earlier (Koebner and Shepherd 1986a). Such derivatives will still possess the *SrR* locus and are less likely to suffer from the quality defect of the parent translocation as they would also

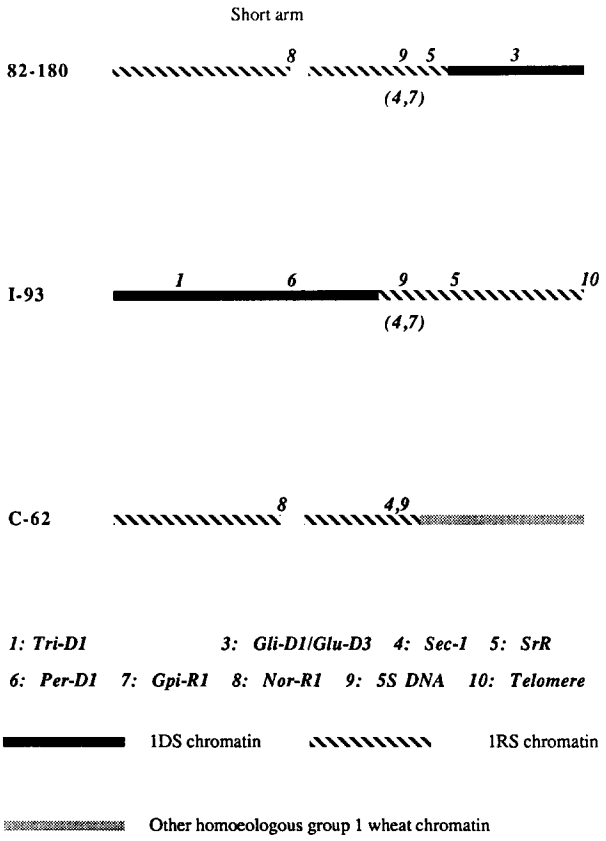

Fig. 8. Physical models of the three wheat-rye recombinant chromosomes in plants 82-180, I-93 and C-62, respectively. Note common regions of 1RS chromatin in the first two recombinant chromosomes

possess both *Tri-D1* and the *Gli-D1/Glu-D3* loci, at least some of which are thought likely to influence dough quality. The recombinant chromosome in plant C-62 possesses *Sec-1* but lacks *SrR,* and since the analysis of molecular markers indicates that the distal portion of 1RS is absent, this establishes that *SrR* is distal to *Sec-1* which confirms the genetic mapping result of Singh (1985). *Sec-1, Gpi-R1* and *5S RNA* remain associated in all three recombinants and thus the relative order of these three loci cannot be established. The gene order along chromosome arm 1RS is therefore *Nor-R1 – (Gpi-R1, Sec-1, 5S RNA) – SrR –* telomere, with all of these loci lying within the satellite of the chromosome.

More genetic markers are required to improve the efficiency with which recombinants can be detected and to refine the analysis of the structure of the recombinant chromosomes already isolated. In particular, markers specific to the rye chromatin are required, as it should be noted that the 1DL-1RS recombinants were selected on the basis of a disruption in the association of wheat markers, and this indirect method is less efficient than the direct observation of disruption of rye markers used in a previous study of wheat-rye recombination (Koebner and Shepherd 1985). Thus it was possible to detect only six wheat-rye recombinants involving 1RS, whereas recombinants involving 1RL were detected relatively more frequently (Koebner and Shepherd 1985). A series of hexokinase (E.C. 2.7.7.1) genes has been reported by Ainsworth (1983) to be located on homoeologous group 1 chromosomes, but no rye isozyme was identified in his study. Recently, a structural gene coding for a grain protease inhibitor was located on chromosome 1R, but arm location was not assigned (Hejgaard et al. 1984); whether this gene is present in the wheat-rye recombinant lines has not yet been determined. No other genes controlling biochemical characters have been located on group 1 chromosomes, and other physiological and morphological genes listed by McIntosh (1983) have no known homoeoloci in rye. Molecular analysis of plant DNA promises to provide a potentially unlimited number of genetic markers by exploiting restriction fragment polymorphisms which appear to be common, at least in maize and to a lesser extent in tomato (Helentjaris et al. 1985), and our work in the present paper shows how related molecular techniques can be applied to the problem of characterisation of allosyndetic recombinants. At present these techniques do not lend themselves to the efficient screening of large progeny populations, and thus are more useful in the characterisation rather than the isolation of rare recombinants.

The short arm of 'Imperial' rye chromosome 1R is reported to be cytologically marked by a satellite (Sybenga 1983), but the satellite is fused with the rest of the arm in hexaploid triticale (Merker 1973) and is also not visible in Feulgen stained somatic chromosome spreads of the 'Veery' 1BL-1RS translocation (Merker 1982). Two heterochromatic bands, one telomeric, the other slightly proximal to the telomere in the nucleolar organising region can be seen in Giemsa C-banded karyotypes of the 'Imperial' rye 1R addition line to wheat cv. 'Chinese Spring' (Darvey and Gustafson 1975) and in 1BL-1RS translocation lines (Bennett and Smith 1975; Merker 1982). However the C-banding of the 1DL-1RS translocation line used in this study failed to reveal the 1RS telomere although a repeated DNA sequence diagnostic for this telomere is still present in this line, as described herein. This difficulty in assaying telomeric heterochromatin by C-banding may represent another example of the modification of rye chromosomes in wheat backgrounds, as suggested for addition lines (Singh and Röbbelen 1976) and triticale (Merker 1975; Lapitan et al. 1984). Loss of a substantial portion of a major rye heterochromatic DNA sequence through normal meiosis, possibly following unequal sister chromatid exchange (Koebner et al. 1986), has been described recently. Reductions in C-band heterochromatin of the short arm telomere have not been reported previously.

It was argued in a previous study of wheat-rye recombination (Koebner and Shepherd 1985) that in a plant heterozygous for the translocation 1DS-1RL and the complete wheat chromosome 1D, it was likely that the alien segment 1RL would lie close to 1DL at meiotic prophase I due to the homologous pairing of the common 1DS arm, and thus in a *ph1bph1b* background, allosyndetic recombination would most frequently involve the production of 1DL/1RL exchanges. A similar scheme was followed in the present work, in order to promote 1DS/1RS exchanges. However the structures proposed for the recombinant chromosomes present in plants 82-177, -178, -179 and -180 (Fig. 6) can only arise from more complex configurations such as trivalents involving three distinct homoeologues, or from bivalents with more than one homoeologous chiasma. The occurrence of such bivalents is unexpected since homoeologous chiasma frequency is likely to be very low. However, direct genetic evidence of these bivalents has been provided in a study of wheat-*Aegilops* allosyndesis (Koebner and Shepherd 1986 b). Thus the observation of wheat-wheat recombinants shows that the disomic state of a given chromosome does not preclude it from becoming involved in allosyndetic pairing with a homoeologue, and this is consistent with the unusually high incidence of rod bivalents and univalents observed in metaphase I pmcs of *ph1bph1b* plants (Koebner and Shepherd 1985).

The recombinant lines isolated in this work will be particularly useful in deducing gene order along 1DS as new markers on this chromosome arm are developed, and the order *Tri-D1 – Per-D1 – Gpi-D1 – Gli-D1* can be obtained in this way from analysis of the recombinants listed in Table 2. The order of the latter three loci agrees with that found on chromosome 1BS by Ainsworth et al. (1984), providing a further example of the conservation of gene synteny resulting from the assumed common ancestry of the individual genomes of wheat. To date the phenomenon of gene synteny conservation has relied purely on chromosome arm location, but this result extends the relationship further to gene order. The 1DS-encoded 5S RNA site reflects the homoeology between chromosomes 1D and 1B, which was already known to possess a 5S RNA site (Appels et al. 1980).

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References

Ainsworth CC (1983) The genetic control of hexokinase isozymes in wheat. Genet Res 42:219–227

Ainsworth CC, Johnson HM, Jackson EA, Miller TE, Gale MD (1984) The chromosomal locations of leaf peroxidase genes in hexaploid wheat, rye and barley. Theor Appl Genet 69: 205–210

Appels R, Dvorak J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. Theor Appl Genet 63:337–348

Appels R, Moran LB (1984) Molecular analysis of alien chromatin introduced into wheat. Stadler Genet Symp 16: 529–557

Appels R, Driscoll CJ, Peacock WJ (1978) Heterochromatin and highly repeated DNA sequences in rye (*Secale cereale*). Chromosoma 70:67–89

Appels R, Gerlach WL, Dennis ES, Swift H, Peacock WJ (1980) Molecular and chromosomal organization of DNA sequences for the ribosomal RNAs in cereals. Chromosoma 78:293–311

Bennett MD, Smith JB (1975) Confirmation of the identification of the rye chromosome in 1B/1R wheat-rye chromosome substitution and translocation lines. Can J Genet Cytol 17:117–120

Chojecki AJS, Gale MD (1982) Genetic control of glucose phosphate isomerase in wheat and related species. Heredity 49:337–347

Darvey NL, Gustafson JP (1975) Identification of rye chromosomes in wheat-rye addition lines and triticale by heterochromatic bands. Crop Sci 15:239–243

Hejgaard J, Bjørn SE, Nielsen G (1984) Rye chromosomes carrying structural genes for the major grain proteases. Hereditas 101:257–259

Helentjaris T, King G, Slocum M, Siedenstrang G, Wegman S (1985) Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–118

Koebner RMD, Shepherd KW (1985) Induction of recombination between rye chromosome 1RL and wheat chromosomes. Theor Appl Genet 71:208–215

Koebner RMD, Shepherd KW (1986a) Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. 1. Isolation of recombinants. Theor Appl Genet 73:197–208

Koebner RMD, Shepherd KW (1986b) Induction of allosyndetic recombination between a chromosome of *Aegilops umbellulata* and wheat chromosomes. Heredity (in press)

Koebner RMD, Appels R, Shepherd KW (1986) Rye heterochromatin II. Characterization of a derivative from chromosome 1DS/1RL with a reduced amount of the major repeating sequence. Can J Genet Cytol (in press)

Lapitan NLV, Sears RG, Gill BS (1984) Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. Theor Appl Genet 68: 547–554

McIntosh RA (1983) A catalogue of gene symbols for wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto, pp 1197–1254

Merker A (1973) Identification of aneuploids in a line of hexaploid triticale. Hereditas 74:1–6

Merker A (1975) Chromosome composition of hexaploid triticale. Hereditas 80:41–52

Merker A (1982) "Veery" – a CIMMYT spring wheat with the 1B/1R chromosome translocation. Cereal Res Commun 10:105–106

Mettin D, Blüthner WD, Schlegel R (1973) Additional evidence on spontaneous 1B/1R wheat-rye substitutions and translocations. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. University of Missouri, Columbia MO, pp 179–184

Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetic studies on the high-molecular-weight subunits of wheat glutenin. 3. Telocentric mapping of the subunit genes on the long arms of the homoeologous group 1 chromosomes. Theor Appl Genet 63:129–138

Payne PI, Jackson EA, Holt LM, Law CN (1984) Genetic linkage between endosperm storage protein genes on each of the short arms of chromosomes 1A and 1B in wheat. Theor Appl Genet 67:235–243

Rajaram S, Mann ChE, Ortiz-Ferrara G, Mujeeb-Kazi A (1983) Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Kyoto, pp 613–621

Righetti PG (1983) Isoelectric focusing: theory, methodology and applications. Elsevier Biomedical Press, Amsterdam New York Oxford, 386 pp

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

Sears ER, Sears LMS (1978) The telocentric chromosomes of common wheat. In: Ramanujam S (ed) Proc 5th Int Wheat Genet Symp. Indian Soc Genet Plant Breeding, New Delhi, pp 389–407

Shepherd KW (1968) Chromosomal control of endosperm proteins in wheat and rye. In: Shepherd KW, Finlay KW (eds) Proc 3rd Int Wheat Genet Symp. Plenum Press, New York, pp 86–96

Shepherd KW (1973) Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. University of Missouri, Columba MO, pp 745–760

Shepherd KW, Jennings AC (1971) Genetic control of rye endosperm proteins. Experientia 27:98–99

Shepherd KW, Singh NK (1984) Yield and quality of wheats carrying a *1DL-1RS* wheat-rye translocation. In: Proc 2nd Workshop on Gluten Proteins (Abstr), Wageningen

Shewry PR, Bradberry D, Franklin J, White RP (1984) The chromosomal locations and linkage relationships of the structural genes for the prolamin storage proteins (secalins) of rye. Theor Appl Genet 69:63–69

Singh NK (1985) The structure and genetic control of endosperm proteins of wheat and rye. PhD Thesis, University of Adelaide, 146 pp

Singh NK, Shepherd KW (1985) The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. Theor Appl Genet 71:79–92

Singh RJ, Röbbelen G (1976) Giemsa banding technique reveals deletions within rye chromosomes in addition lines. Z Pflanzenzücht 76:11–18

Sybenga J (1983) Rye chromosome nomenclature and homoeology relationships. Z Pflanzenzücht 90:297–304

Wehling P, Schmidt-Stohn G, Wricke G (1985) Chromosomal location of esterase, peroxidase and phosphoglucomutase isozyme structural genes in cultivated rye (*Secale cereale* L.). Theor Appl Genet 70:377–382

Zeller FJ (1973) 1B/1R wheat-rye substitutions and translocations. In: Sears ER, Sears LMS (eds) Proc 4th Int Wheat Genet Symp. University of Missouri, Columbia MO, pp 209–221

Zeller FJ, Fuchs E (1983) Cytologie und Krankheitsresistenz einer 1A/1R und mehrerer 1B/1R Weizen-Roggen-Translokationssorten. Z Pflanzenzücht 90:285–296

Zeller FJ, Gunzel G, Fischbeck G, Gerstenkorn P, Weipert D (1982) Veränderung der Backeigenschaften der Weizen-Roggen-Chromosomen-Translokation 1B/1R. Getreide Mehl Brot 36:141–143