Theor Appl Genet (1999) 98: 647–656

U. Hohmann · J. Zoller
R. G. Herrmann · M. E. Kazman

Physical mapping and molecular-cytogenetic analysis of substitutions and translocations involving chromosome 1D in synthetic hexaploid triticale

Received: 27 July 1998 / Accepted: 5 August 1998

Abstract Chromosome 1D, which carries the advantageous alleles of glutenin and gliadin, attracts major interest with respect to improving the bread-making quality of triticale. Eighty-one BC_1F_4 lines from different primary and secondary hexaploid triticale crosses were selected for 1D chromatin analysis using SDS-PAGE and C-banding. In situ hybridization and RFLP-based comparative physical mapping of group 1 chromosomes revealed 20 lines with complete 1D (1A) substitutions. Nine 1D (1B) substitutions, six 1D (1R) substitutions and one 1D addition line were also selected. Three lines were pure AABBRR hexaploids without any D-genome chromosomes. For the remaining 42 lines (51.8%), a wide spectrum of 20 different recombinations between chromosomes 1A and 1D was uncovered. Altogether, they were generated without any earlier irradiation, tissue culture or genetic induction of chromosome pairing. In addition, 14 translocations between 1B/1D, 1A/1R, 1B/1R, 1D/1R and 1A/1B were detected. Considerable variability for sedimentation values was found, with the highest sedimentation values among lines with complete 1D chromosomes. The implications of using triticale as a model for generating compensating chromosome rearrangements in defined homoeologous groups and the breeding potential of D-genome chromatin introgressed into triticale with improved sedimentation values are discussed.

Key words Physical map · Synteny · FISH · C-banding · Homoeologous recombination

Communicated by G. Wenzel

U. Hohmann (✉) · J. Zoller · R. G. Herrmann
Botanisches Institut der Ludwig-Maximilians-Universität München, Menzinger Str. 67, D-80638 München, Germany
Fax: + 49-89-178 22 74
E-mail: uhemail@botanik.biologie.uni-muenchen.de

M. E. Kazman
Institut für Pflanzenbau und Pflanzenzüchtung, Georg-August Universität Göttingen, Von-Siebold-Str. 8, D-37075 Göttingen, Germany

Introduction

Hexaploid triticale, a man-made wheat-rye hybrid (AABBRR) between tetraploid wheat (*Triticum durum* Desf.) and diploid rye (*Secale cereale* L.), is increasingly cultivated worldwide due to its relatively high yield and satisfactory performance under marginal environments. However, these forms are still of limited use for human consumption due to their inferior bread-making quality compared to hexaploid wheat (AABBDD) with the D genome of *Triticum tauschii*. There is considerable worldwide interest in improving the quality of triticale for breadmaking and special bread-like products.

The D genome of wheat with its valuable spectrum of genes has been widely used for the improvement of quality, disease resistance, winter hardiness and other characteristics of hexaploid triticale (AABBRR). In wheat/triticale crosses the D-genome chromosomes are substituted for rye chromosomes. In octoploid × tetraploid triticale progenies, the D-genome chromosomes substitute for their wheat A- or B-genome chromosomes, and the rye genome remains complete. A set of hexaploid triticale lines has been established, in which chromosome 1D is present in different combinations, i.e., the disomic substitutions 1D (1A), 1D (1B) and 1D (1R) substitutions as well as the disomic 1D addition (Kazman and Lelley 1994).

Chromosome 1D is the most desirable chromosome for the improvement of the gene pool for several reasons: (1) it carries genes of interest i.e., allelic variation at the *Glu-D1* locus on chromosome 1D has been reported to play a major role in the bread-making quality of bread wheat (Payne et al. 1987), (2) it substitutes for chromosomes 1A and 1B at good and almost

equal frequencies, (3) it has little effect on fertility reduction and (4) it has a good compensating ability in primary triticale (Lukaszewski et al. 1987; Hohmann 1988). The high frequency of chromosome 1D in triticale (Kazman and Lelley 1994) and that of the translocation involving 1D (Kazman et al. 1996) is caused by the conserved homoeology and collinearity within the homoeologous group 1 chromosomes (Hohmann and Kazman 1998). The chromosome 1R is evidently the only non-translocated rye chromosome relative to that of hexaploid wheat (Devos et al. 1993; Hohmann and Kazman 1998).

To further improve the characteristics of synthetic hexaploids with D-genome chromatin and to find out whether chromosome 1D or its segments can be transferred into different genetic backgrounds we carried out crosses involving the 1D substitution lines, 8x triticale and pure AABBRR triticale cultivars. The objectives were: (1) to substitute the inferior *Glu-D1* alleles coding for the high-molecular-weight (HMW)-glutenin subunits 2+12 with the alleles coding for 5+10, which confers high dough strength, (2) to induce variability for increasing falling number and decreasing their pentosan content and (3) to develop near-isogenic lines to exploit whether the 5+10 allele can further improve bread-making quality in triticale.

We report here on the cytogenetic stability or instability associated with the introduction of chromosome 1D into hexaploid triticale. New 1D substitutions and a vast population of recombinant group 1 chromosomes were selected from different triticale crosses without the necessity of irradiation, tissue culture or induction of chromosome pairing. The frequencies of homoeologous recombination and centric or near-centric breakage-fusion translocations are discussed with respect to aspects of practical breeding.

Materials and methods

Materials

Eighty-one triticale lines were selected using SDS-PAGE to possess 1D chromatin and analyzed in the present study. Sixty-four of the synthetic triticale (BC_1F_4) lines were obtained from crosses involving primary 6x synthetic 1D (1A, 1B or 1R) substitutions or 1D additions and (1) 6x triticale cvs 'Lasko', 'Clercal' and 'Alamo' (38 lines) or (2) 8x triticale (26 lines). The primary 6x synthetic triticale lines originated from the crosses (8x × 4x) × 8x triticale (Kazman and Lelley 1994). Seventeen new primary hexaploids were selected from (8x × 4x) × 8x triticale crosses. Their pedigree is shown in Table 1.

SDS-PAGE

Reduced storage protein of synthetics and triticale cultivars was analyzed using the procedure described by Galili and Feldman (1981). Four or five seeds of each line that were determined to carry genes of chromosome 1D were analyzed in every generation to determine the doses of 1D chromatin. Glutenin analysis for the parents has been described earlier (Kazman et al. 1996).

Table 1 Pedigree of 81 new synthetic triticale lines selected from crosses between primary synthetic triticale lines carrying 1D (1A, 1B or 1R) substitutions or 1D additions and three triticale cultivars ('Alamo', 'Clercal' and 'Lasko'): (a) cultivars × primary synthetics, (b) (primary synthetics × 8x) × cultivar and (c) new primary triticale selected from crosses (8x × 4x) × 8x triticale

Cross cultivar × synthetic	Primary synthetic	Chromosome constitution of primary synthetic	Line (no.)
a)			
6x × 6x synth	K30-5	1D (1A)	1, 2, 3, 4, 5, 6, 7, 8
	M92	1D (1A)	36, 37, 38
	KT642-14	1D (1A), 5D (5A)	29, 30, 31, 32, 33, 34, 35
	KT49-10	1D (1A), 3D (3A), 7D (7B)	22, 23, 24, 25, 26, 27, 28
	K39-4	1D (1B)	9
	K921-6	1D (1B)	21
	K21-10R	1D (1R)	10, 11, 12, 13, 14, 15, 16
	K21-10D	1D addition	17, 18, 19, 20
Subtotal			38
b)			
6x × (8x × 6x synth)	K30-5	1D (1A)	45, 46, 47, 48, 49, 50
	K38-1	1D (1A)	39, 40, 41, 42, 43, 44 51, 52, 53, 54, 55, 56, 57
8x × 6x synth	K30-5	1D (1A)	58, 59, 60, 61
	K38-1	1D (1A)	62, 63, 64
Subtotal			26
c)			
(8x × 4x) × 8x		?	65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81
Subtotal			17
Total			81

C-banding, fluorescent in situ hybridization (FISH) and genomic in situ hybridization (GISH)

Prior to crossing, mitotic metaphase chromosomes of five to six seedlings of the synthetic triticale were analyzed using the C-banding method to identify 1D chromosomes or chromosome arms in early generations (Lukaszewski and Gustafson 1983). From the primary synthetic triticale involved in the crossing, five lines carried a 1D (1A) substitution, two lines a 1D (1B) and one line a 1D (1R) substitution. One line possessed an 1D addition. The FISH and GISH analysis were performed according to Busch et al. (1995) and Hohmann et al. (1996). Some lines were analyzed in BC_1F_4 by GISH to verify the complete set of 14 rye chromosomes. Entire genomic DNA of rye was labelled with Cy3 and hybridized in the presence of a 100-fold excess of unlabelled sonicated wheat DNA. FISH analysis was performed with the D-genome-amplified sequences pAs1 (Rayburn and Gill 1986) and with an satellite DNA sequence pHvMWG2314 (Busch et al. 1996) to verify complete 1D substitutions or translocations involving chromosome 1D.

Restriction fragment length polymorphism (RFLP)

Eighty-one synthetic triticale lines were analyzed by RFLP analysis in the BC_1F_4 generation. The extraction of genomic DNA, DNA digestion with *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, or *Hin*dIII restriction endonucleases, transfer onto Nylon membranes, random priming and hybridization have been described earlier (Hohmann and Kazman 1998). RFLP probes were generously provided by Dr. M. D. Gale, Norwich (designated PSR), Dr. K. S. Gill, Kansas (designated KSU), Dr. M. E. Sorrells, Ithaca (designated BCD), and Dr. A. Graner, Grünbach (designated MWG). The mapped loci were designated *Xpsr*, *Xksu*, *Xbcd*, and *Xmwg*, respectively. A cytogenetically-based physical map of the group 1 chromosomes of common wheat was constructed by utilizing 14 homozygous deletion stocks of 'Chinese Spring' (CS) (Endo and Gill 1995, 1996) and 32 RFLP markers. The procedure for constructing the physical map was done essentially as described in Werner et al. (1992).

Results

Physical mapping of homoeologous group 1 loci

For physical mapping, we selected 32 RFLP fragments that derived from wheat, barley, oat or *Triticum tauschii*, and had been previously used for linkage mapping. We focussed on (1) evenly distributed markers to cover most of the chromosome arms, (2) markers that were clustered around the centromere of the linkage map and (3) markers that revealed polymorphism for all four genomes, A, B, D and R, of triticale. Of the 32 probes selected, a total of 14 identified loci were used to physically map the wheat chromosomes. For the remaining 18, no clear polymorphism in the individual genomes of wheat and rye was detected with 6 probes from wheat (PSR158, PSR596, WG180, KSUD49, KSUE8 and KSUE18), and the other 12 fragments derived from barley (cMWG733, MWG896, MWG912, MWG913, MWG938, MWG943, MWG2261, BCD-310, BCD1150 and BCD1261) and oat (CDO99 and CDO534) showed no hybridization under high stringency conditions.

The majority of the probes, 10 out of 14 (71.4%), detected polymorphic loci in all three genomes of hexaploid wheat; 5 of these were polymorphic for the rye genome as well (Table 2). The remaining 4 probes (PSR78, KSUG2, KSUG9 and BCD1434) could only be used for mapping one or two genomes because of the lack of corresponding fragments in the complementing genomes. For some markers, for example PSR544 and PSR162, polymorphism was detected for the B and R genome of the different hexaploid cultivars and octoploid triticale (Fig. 1). Therefore, the individual parental chromosomes 1B and 1R could be distinguished in the new synthetic triticale. The short arm markers often existed in a duplicated form. For rye, the short arm 'specific' band often cosegregated with one of the signals from the wheat genomes. Therefore, not all of the loci physically mapped in wheat could be applied in mapping triticale, and most of the polymorphic markers were from the long arm.

Physical maps of chromosomes 1A, 1B and 1D and comparison with linkage maps

Of the 14 RFLP markers, 11 had not been previously used to physically map particular chromosome arm regions in wheat. These mapped loci provided a reasonable physical coverage of proximal, interstitial and distal regions of group 1 chromosomes of wheat (Fig. 2). For chromosome 1A, three short arm and two long arm chromosome regions were tagged by 13 molecular markers. The proximal segment (FL 0-0.17) remained untagged. For chromosome 1B, six deletion lines were examined, including three deletions each for both the long and short arm. Seven molecularly tagged chromosome regions (MTCRs) were established with 10 RFLP markers. For chromosome 1D, four deletion lines were analyzed, two for each arm. All six regions were tagged using 13 RFLP and two protein markers. For 1 short arm marker (*Xpsr688*) and 1 long arm marker (*XksuE11*) duplicated loci were detected. Despite the fact that chromosomes 1A, 1B and 1D differ in size and amount and distribution of heterochromatin, the relative position of physically mapped homologous loci was conserved and collinear with genetic mapping data.

Physical mapping of group 1 chromosomes in triticale

Restriction fragments specific for chromosome regions were detected for the A-, B-, D- and/or R-genome chromosomes of homoeologous group 1 in 81 triticale lines. The presence of RFLP markers specific for D-chromosome regions and the absence of A-, B- or R-specific markers were an indication for a substitution or translocation involving chromosome 1D. There were no clear differences in the frequencies of

Table 2 Origin, number and polymorphisms detected with DNA fragments used for physical mapping of group 1 chromosomes of wheat

Clone designation[a]	Origin	Type	Number of clones	Number of polymorphism			
				A	B	D	R
PSR161[b], PSR162[a], PSR544[a], PSR601[a], PSR688[b], PSR949[a], PSR953[c],PSR957[a]	*T. aestivum*	cDNA	8	8	8	8	4
KSUE11[a]	*T. tauschii*	genomic DNA	1	1	1	1	0
cMWG706[c]	*H. vulgare*	cDNA	1	1	1	1	1
Subtotal			10	10	10	10	5
PSR78[a]	*T. aestivum*	cDNA	1	0	0	1	0
KSUG2[d], KSUG9[b],	*T. tauschii*	genomic DNA	2	1	1	1	0
BCD1434[c]	*H. vulgare*	cDNA	1	1	0	0	0
Subtotal			4	2	1	2	0
Total	RFLP		14	12	11	12	5

[a] Restriction endonucleases used for detection of RFLPs were a, *Dra*I; b, *Hin*dIII; c, *Eco*RV; d, *Eco*RI

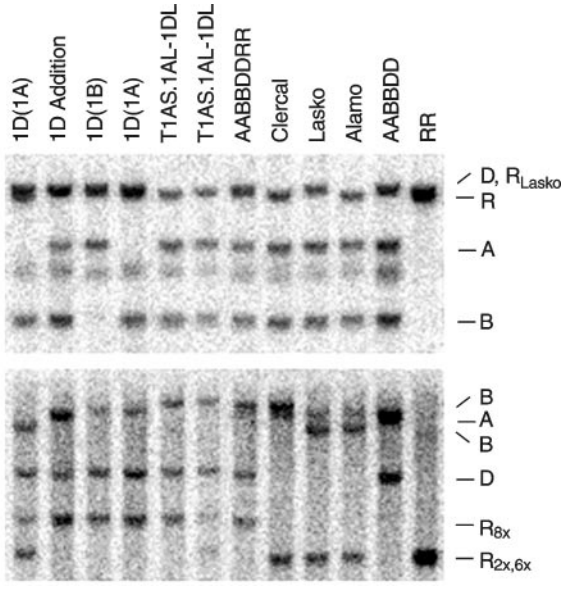

Fig. 1 Hybridization to genomic DNA using the wheat cDNAs PSR544 and PSR162. DNA of secondary synthetic triticale lines 76 (1D (1A)), 77 (1D addition), 78 (1D (1B)), 79 (1D (1A)), 74 (T1AS·1AL-1DL), 75 (T1AS·1AL-1DL), 8x triticale (AABBDDRR), 6x triticale cvs 'Clercal', 'Lasko', 'Alamo', *Triticum aestivum* cv 'Rektor' and *Secale cereale* cv 'Locarno' was digested with restriction endonuclease *Dra*I. The chromosome origin of the bands was determined from nullitetrasomic lines of *T. aestivum* cv 'Chinese Spring'. Note: The R (rye) and B genome in the parental 6x and 8x triticale show RFLPs, which allow the discrimination of individual wheat and rye chromosomes of different origin in secondary synthetic triticale

substitutions and translocations between the crosses and different triticale cultivars that were used. Therefore, the data were pooled. In general, the chromosome substitution pattern of the lines determined by C-banding and SDS-PAGE could be confirmed. Lines with possible translocations involving chromosome arms 1DS or 1DL could be identified as subarm-chromosomal translocations. The RFLP analysis and deletion mapping of 4 lines revealed refined results with an 1D addition (line 23), 1D (T1BS·1BL-1AL) substitution and possibly 1AS·1RL (line 42) or 1RS-1DS·1DL-1RL translocations (line 66). Refined analysis, especially with additional short arm markers, could detect more chromosome rearragements.

In the 81 progenies of secondary triticale that were analyzed, 20 lines with complete 1D (1A) substitutions were detected by the comparative physical mapping of group 1 chromosomes (Table 3). The RFLP analysis uncovered restriction fragments specific for chromosomes 1B and 1D. The A-genome-specific fragments were absent. Nine of the lines carried 1D (1B) substitutions, 6 lines 1D (1R) substitutions and 1 line possessed an 1D addition. Three lines were pure AABBRR hexaploids without any D-genome chromosomes. Regardless of the chromosome constitution of the parental primary triticale used in crossing, more than 50% of the lines (42 of 81 lines, 51.8%) possessed a translocated chromosome involving 1D. Among 42 lines, 20 different translocated chromosomes concerning 1D and 1A were detected, including 12 reciprocal translocations between the most distal regions of the short and/or long arms (Table 4). Eight recombinants with intercalary translocations proved pairing and chromatin exchange between group 1 homoeologues in triticale. The T1DS·1DL-1AL-1DL translocation, a chromosome 1D with a small intercalary subterminal chromosome segment from 1A, occurred most frequently and was detected in the genetic background of all three triticale cultivars (Fig. 3). Their appearance in different crossing combinations indicated the independent nature of origin. In addition, 5 translocations between 1B/1D, 1D/1R, and 9 between 1A/1R, 1B/1R, and 1A/1B with

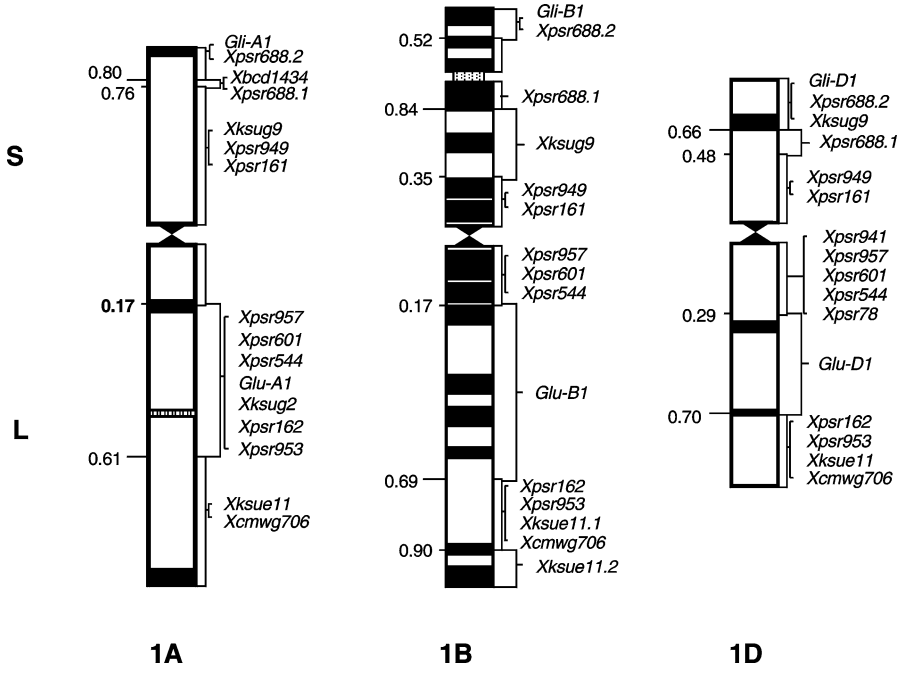

Fig. 2 Physical maps of C-banded wheat chromosomes 1A, 1B and 1D. Fractional length (FL) measurements are shown on the *left* and marker allocation on the *right*

Table 3 Frequency of complete and translocated 1D chromosomes in progenies of secondary synthetic triticale obtained from crosses of hexaploid or octoploid triticale with primary synthetic triticale containing 1D (1A, 1B or 1R) substitutions or 1D additions as well as from 8x × 4x crosses

Primary synthetic parent	Progeny of secondary synthetics (no. of lines)						
	Substitutions			Additions	Pure AABBRR	Translocations	Total
	1D (1A)	1D (1B)	1D (1R)	1D			
1D (1A)	15	2	2	1	2	26	48
1D (1B)	–	2	–	–	–	–	2
1D (1R)	2	–	2	–	1	5	10
1D addition	–	–	2	–	–	2	4
8x × 4x	3	5	–	–	–	9	17
Total	20	9	6	1	3	42	81

translocation breakpoints at or near the centromere were observed. The presence of multiple recombinant chromosomes demonstrates the possibility that homoeologous chromosomes recombine in a hexaploid triticale background.

The recombination (translocation) frequency between chromosome arms of 1D and 1A was higher than between chromosome arms of 1D and 1B or 1R, respectively. In the 48 progenies of 1D (1A) substitutions 31 recombination events for the short arm (0.64 per line) and 47 for the long arm (0.98 per line) were determined. In the offspring of 1D (1B) substitutions no structural chromosome modifications were observed. The 1D (1R) substitution or 1D addition lines could be transferred into different genetic backgrounds. Two lines with 1D (1A) substitutions and several centric-break translocations involving chromosome arms 1AS, 1BS and 1RS were identified. In the progenies of the crosses involving the 1D addition lines, two A/D translocations were obtained. However, the number of chromosomes with proximal or interstitial translocation breakpoints was much lower than when the 1D (1A) substitution was used in the crosses. In the progenies of the 8x × 4x crosses the substitution frequency of translocated and non-translocated chromosomes was almost identical. Again, recombination occurred preferentially between wheat chromosomes 1A and 1D (Table 3).

Table 4 Detection of 1D (1A) substitutions and recombinants (translocations) by means of the presence of D-genome-specific RFLP or protein marker(s) substituting for A- or B-genome-specific marker(s)

Chromosome constitution	1DS, distal		1DS, proximal			1DL, proximal		1DL, interstitial		1DL, distal				Number of lines
	Gli-1	PSR 688.2	PSR 949	PSR 161	PSR 957	PSR 601	PSR 544	PSR 78	Glu-1	PSR 162	PSR 953	KSU E11.1	MWG 706	
1D (1A)	++	++	++	++	++	++	++	++	++	++	++	++	++	20
1D (1A) single recombinants	++	++	++	++	++	++	++	++	++	++	++	−−	−−	1
	++	++	++	++	++	++	++	++	+−	−−	−−	−−	−−	1
	++	+−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	1
	++	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	1
	−−	++	++	++	++	++	++	++	++	++	++	++	++	2
	−−	−−	−−	−−	−−	−−	−−	−−	++	++	++	++	++	2
	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	++	3
1D (1A) double recombinants	++	++	−−	−−	−−	−−	−−	−−	−−	−−	++	++	++	1
	−−	+−	−−	−−	−−	−−	−−	−−	−−	−−	++	++	++	1
	++	++	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	+−	1
	++	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	−−	++	1
	++	−−	−−	−−	−−	−−	−−	−−	−−	+−	+−	+−	++	2
1D (1A) intercalary recombinants	−−	+−	−−	−−	−−	−−	−−	−−	−−	++	++	++	++	1
	−−	+−	−−	−−	−−	−−	−−	−−	++	++	+−	+−	++	1
	++	++	++	++	++	++	++	++	++	++	++	−−	++	8
	++	+−	++	++	++	++	++	++	++	++	++	++	++	1
1D (1A) double and intercalary recombinants	−−	++	++	−−	++	−−	++	−−	++	++	−−	++	+−	1
	−−	++	++	−−	++	++	++	+−	++	++	++	−−	+−	1
	−−	++	−−	++	−−	++	++	+−	++	++	++	++	++	1
	−−	++	++	++	++	++	++	−−	++	++	++	−−	++	1

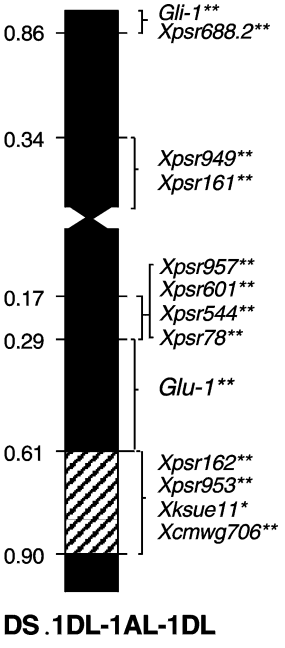

Fig. 3 Physical map of the most frequently detected 1DS·1DL-1AL-1DL translocation in synthetic hexaploid triticale. Chromosome markers specific for 1A (*) and 1D (**) are indicated

RFLP analysis revealed the duplication and deficiency of several markers, which indicates the occurrence of unequal crossing-over events. In general, RFLP loci of the wheat component occurred in duplicated constellation, whereas loci of the rye genome were preferentially eliminated from the triticale genome. In total, 29 duplicated loci specific for chromosome 1A, 14 short arm and 15 long arm markers, were detected. In addition, 18 duplicated loci specific for chromosome 1D were determined. Deficiency of markers, which may indicate the dispensable character of chromosome regions, occurred in much lower frequencies than duplications. In addition, several other translocations between 1A/1B, 1A/1R were detected. In the sample of plants analyzed, 34 accessions were heterozygotes or still segregating for one or the other homozygote.

SDS-PAGE

Seeds from 81 lines of crosses between synthetic triticale with 1D (1A, 1B or 1R) substitution, 1D addition, crosses between $(8x \times 4x) \times 8x$ triticale and three triticale cultivars 'Alamo', 'Clercal' and 'Lasko' were analyzed. The doses of chromosome 1D were determined in the F_4 progeny (Table 5). HMW-glutenin subunits encoded by *Glu-D1* on 1DL as well as the gliadin encoded by *Gli-D1* on 1DS could be identified unambiguously and confirmed the chromosome constitution of all lines determined by physical mapping.

C-banding

The chromosome constitution obtained from the C-banding analysis of 77 lines in BC_1F_3 was in agreement with the data of the physical mapping using 81 lines in BC_1F_4. For 4 lines refined results were obtained. The progeny of a 1D (1A) substitution (line 23), a 1D addition, occurred based on physical mapping. In line 78, the 1BS·1BL-1AL translocation was substituted by 1D. Line 42 carries a possible 1AS·1RL, line 66 a 1RS-1DS·1DL-1RL translocation. Twenty-four lines that were preliminarily determined as centric-break translocations by C-banding were proven to carry translocations, preferentially in interstitial and proximal regions. The low number of polymorphic C-bands of chromosome 1A and 1D did not allow a more detailed analysis.

FISH and GISH analysis

The presence of centric-break translocations was tested with FISH using the pAs1 and the pHvMWG2314 sequences as probes. The D-genome-specific, highly repeated DNA sequence pAs1 generates characteristic hybridization sites in the distal parts of 1DL and 1DS but not on 1AL. A minor, distal signal was observed on 1AS. Therefore, chromosome arm recombinants between 1A and 1D could be detected. The satellite DNA pHvMWG2314 causes a prominent signal in the proximal region of chromosome arm 1DL. However, the positions of the long arm signals on 1DL from pHvMWG2314 and pAs1 were almost identical to the position of the C-bands of 1DL (data not shown). Therefore, with the DNA markers currently available

Table 5 Synthetic triticale lines with chromosome 1D (monosome/disome) and gliadin encoded by *Gli-D1* on 1DS as well as the HMW glutenin subunits encoded by *Glu-D1* on 1DL in BC_1F_4

Chromosome constitution/cross	*Gli-D1*			*Glu-D1*			Number of lines
	Disome	Monosome	Nullisome	Disome	Monosome	Nullisome	
1D (1A)	35	8	8	37	6	8	48
1D (1B)	2	–	–	2	–	–	2
1D (1R)	5	–	2	5	–	2	10
1D addition	4	–	–	2	–	2	4
$(8x \times 4x) \times 8x$	13	2	2	14	1	2	17

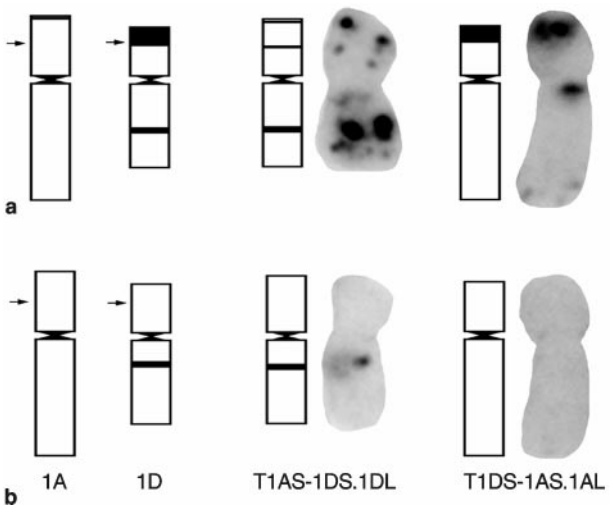

Fig. 4a, b Ideograms and FISH patterns of reciprocal translocation chromosomes T1AS-1DS·1DL and T1DS-1AS·1AL detected in line 41 using repetitive DNA sequences pAs1 **a** and pHvMWG2314 **b** as probes. Unmodified chromosomes 1A and 1D are shown on the *left*. The possible breakpoints are depicted by *arrows*

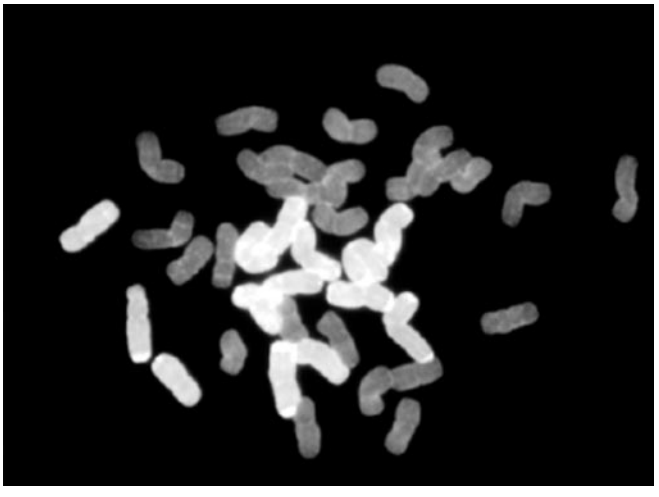

Fig. 5 GISH analysis of hexaploid triticale line 3 with 14 labelled *Secale cereale* chromosomes

for FISH, no additional chromosome rearrangements could be observed. Consequently, a small number of lines were analyzed. For example, in line 41 the reciprocal translocation between the most distal parts of chromosome arm 1AS and 1DS could be identified (Fig. 4). In addition, the presence of the complete rye genome was detected with GISH, as was the labelled total genomic DNA of rye in all 11 triticale analyzed by in situ hybridization so far (Fig. 5).

Discussion

The objective of this study was to determine the nature of the substitution and translocation frequencies of chromosome 1D in offsprings of crosses between synthetic triticale carrying 1D associated with improved baking quality and triticale cultivars. The relatively large number of lines produced enabled us to test whether complete 1D (1A), 1D (1B), 1D (1R) substitutions or 1D additions can be efficiently transferred into different genetic backgrounds. In addition, it was determined whether the effect of a single chromosome can be studied and evaluated by the transfer of single substitutions of D-genome chromosomes into uniform genetic backgrounds.

Mapping of group 1 chromosomes

Genetic mapping had revealed the collinearity of genes across cereal genomes (Ahn and Tanksley 1993; Ahn et al. 1993). The collinearity of physical maps of wheat chromosomes has been shown to be effective for the characterization of recombinant chromosomes (Hohmann et al. 1996; Nasuda et al. 1998). The physical map of chromosome 1B (Kota et al. 1993) was extended with 14 molecular markers specific for group 1 chromosomes, and all markers were collinear with the order on 1A and 1D. Restriction fragments specific for chromosome regions were detected for the A-, B-, D- and/or R-genome chromosomes of homoeologous group 1. The presence of regions specific for the D chromosome detected by RFLP marker technology, for example, and the absence of A-, B- or R-specific markers were applied to detect complete and recombinant group 1 chromosome arms at high resolution and prove the nature of substitution and translocation frequencies of chromosome 1D in BC_1F_4 generations of secondary synthetic triticale.

Introduction of chromosome 1D

The high frequency of chromosome 1D in the progenies of various substitution lines supports the observation that chromosome 1D has a good compensating ability in hexaploid triticale (Lukaszewski et al. 1987; Hohmann 1988) and that there is no or little selection pressure against the chromosome. However, among the 81 lines analyzed, 34 accessions were heterozygotes or still segregating for one or the other homozygote, a phenomenon that has been observed frequently during the process of the substitution of 1RS·1AL and 1RS·1BL translocations in wheat (Lukaszewski 1990) and D-genome introgression into tetraploid triticale (Hohmann and Krolow 1991). The high number of heterozygotes may indicate the presence of additional centric-break translocations or non-compensating translocations which are retained in the population and are not clearly detectable with the deletion mapping or cytogenetic banding techniques. The RFLP analysis revealed the duplication and deficiency of several

markers, which indicates the occurrence of unequal crossing-over events. In total, 29 and 18 duplicated loci specific for chromosome 1A and 1D, respectively, were detected. Deficiency of markers, which may indicate the dispensability of the respective chromosome regions, occurred in much lower frequencies than duplications.

1D recombinants

The relatively high frequency of 1A/1D recombinants that was mostly undetectable by cytogenetic or protein analysis indicates a high pairing frequency between the two homoeologues 1A and 1D. These two chromosomes are similar in size and possess, therefore, one prerequisite for pairing initiation. Naranjo et al. (1988) showed that the long arms of these chromosome have the highest pairing affinity in wheat. Lukaszewski and Curtis (1994) selected two series of translocations involving the *Glu-D1* gene from 1D and chromosome 1A. In the present study, the double monosomic 1A/1D chromosome constitution in F_1 hybrids of triticale is obviously sufficient to induce the pairing and recombination necessary to promote gene transfer across homoeologues.

In polyploid grasses, the most effective gene transfers from alien species into wheat are by complete chromosome substitutions or by compensating recombination of homoeologous chromosomes. For the improvement of the gene pool of wheat, different strategies have been applied successfully, for example irradiation-induced chromatin transfer, tissue culture-induced somaclonal variation and homoeologous recombination-induced suppression of homologous pairing or induction of homoeologous pairing. In hexaploid wheat, pairing between homoeologues is controlled by the *Ph* system. The selection of *Ph* mutants or the use of 5D (5B) substitutions allowed the design of a more precise introgression of alien chromatin into wheat. In the present study it was proven on the molecular level that in triticale the *Ph* system is of minor significance for chromosome pairing, as suggested by Lukaszewski and Curtis (1994), and homoeologous pairing is frequent (Lukaszewski and Curtis 1992, 1994). The hexaploid triticale may serve as an alternative model to induce chromosome recombination between homoeologues without the necessity of additional induction or suppression of chromosome pairing.

The distribution of crossing over and, therefore, recombination (and translocation) was primarily restricted to the distal part of the chromosomes. This is in agreement with suppressed recombination in proximal chromosome regions and with strong chiasmata interference (Curtis and Lukaszewski 1992; Lukaszewski 1995). However, the detection of a limited number of double or multiple recombinants in the present study shows that chiasma interference can be lower between homoeologues or be reduced by structural chromosome rearrangements.

Impact on plant breeding

The results of this study have revealed that chromosome 1D can be transferred to triticale cultivars and regenerate a new genetic background. However, the transfer of chromosome 1D into a different genetic hexaploid triticale background had a negative influence on the sedimentation value in some lines. Kazman and Lelley (1996) reported that in the breeding of hexaploid triticale with improved sedimentation values it is essential to transfer the complete chromosome 1D. The lines developed here were backcrossed to triticale cultivars. Although backcrossing improves agronomic performance, especially seed set and seed quality, often these two characters are negatively correlated. This could be one reason for the reduced sedimentation values of some lines (Hohmann et al. 1998). On the other hand, the genetic background of the new secondary synthetics could not be appropriate for chromosome 1D. The application of molecular markers, in addition to SDS-PAGE and C-banding, proved the presence of considerable chromosome rearrangements between 1D and 1A which resulted in translocation, duplication and deficiencies. Karyotype analysis showed that lines with higher sedimentation values had substitutions of complete 1D chromosomes or a 1DS·1DL-1AL-1DL translocation chromosome with a small intercalary subterminal chromosome segment from 1A. The lines with lower sedimentation values had recombined chromosomes with multiple rearrangements.

For breeding, the chromosome modifications have to be stabilized and/or eliminated by selection in the following generations. It should be possible not only to select lines with complete 1D chromosomes but also chromosome translocations with small segments of chromosome 1D. Therefore, the present material may have a valuable breeding potential to further improve the bread-making quality and agronomic performance of synthetic triticale. Lukaszewski et al. (1987) suggested that some D-genome chromosomes may have a poor compensating ability and/or other undesirable effects in triticale, which can be overcome by inducing centric-break and fusion translocations or crossing over between A-, B- and D-genome chromosomes. The high frequency of recombination between homoeologues and, therefore, the probability for cytogenetic instability may have an impact on the development of isogenic lines in triticale. Every backcross induces heterologous chromosome pairs for group 1 but may also do so for other homoeologous groups. Homoeologous pairing and recombination recovers gametes with new recombined chromosomes which may segregate randomly and reduce the probability for the selection of isogenic lines.

The variation in the crossing scheme can preferentially aid selection either for centric-break translocations involving whole chromosome arms or for terminal or interstitial translocations between two homoeologous wheat or wheat and rye chromosomes. The present data suggest to work either with 1D (1B) and 1D (1R) substitutions to transfer complete chromosomes or chromosome arms of 1D or to use 1D (1A) substitutions to transfer chromosome segments of it, respectively, in order to develop new karyotypes of tetraploid wheat, hexaploid wheat or triticale with different doses of favourable glutenin and gliadin alleles and improved baking quality, winter hardiness and disease resistance.

Acknowledgements We thank M. D. Gale, K. S. Gill, M. E. Sorrells and A. Graner for providing wheat and barley RFLP probes. The generous availability of the genetic mapping data by M. D. Gale and K. Devos is appreciated. The technical assistance of A. Neumann is gratefully acknowledged. The deletion lines were kindly provided by Dr. T. R. Endo and Dr. B. S. Gill. This research was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG; grant No Ho-1217) and partly by the private plant breeders. The experiment complies with current laws of the country.

References

Ahn SN, Tanksley SD (1993) Comparative linkage maps of the rice and maize genomes. Proc Natl Acad Sci USA 90:7980–7984

Ahn SN, Anderson JA, Sorrells ME, Tanksley SD (1993) Homoeologous relationships of rice, wheat and maize chromosomes. Mol Gen Genet 241:483–490

Busch W, Martin R, Herrmann RG, Hohmann U (1995) Repeated DNA sequences isolated by microdissection. I. Karyotyping of barley (*Hordeum vulgare* L.). Genome 38:1082–1090

Busch W, Herrmann RG, Hohmann U (1996) Repeated DNA sequences isolated by microdissection. II. Comparative analysis in *Hordeum vulgare* and *Triticum aestivum*. Theor Appl Genet 93:164–171

Curtis CA, Lukaszewski AJ (1992) The effect of colchicine on the distribution of recombination and chiasma interference in wheat. In: Hoisington D, McNab A (eds) Progress in genome mapping in wheat and related species. (Proc 3rd Public Workshop Int Triticeae Mapping Initiative.) El Batan, Mexico, pp 1–2

Devos KM, Atkinson MD, Chinoy CN, Francis HA, Harcourt RL, Koebner RMD, Liu CJ, Masojc P, Xie DX, Gale MD (1993) Chromosomal rearrangements in the rye genome relative to that of wheat. Theor Appl Genet 85:673–680

Endo TR, Gill BS (1995) Production of deletion stocks in common wheat. In: Li ZS, Xin ZY (eds) Proc 8th Int Wheat Genet Symp. Agricultural Scientech Press, Beijing, vol 1, pp 211–216

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Galili G, Feldman M (1983) Genetic control of endosperm proteins in wheat. 1. The use of high resolution, one-dimensional gel electrophoresis for the allocation of genes coding for endosperm protein subunits in the common wheat cultivar 'Chinese Spring'. Theor Appl Genet 64:97–101

Hohmann U (1988) Direct use of hexaploid wheat in production of hexaploid triticale. In: Miller TF, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp. Bath Press, Bath, UK, pp 303–308

Hohmann U, Kazman ME (1998) Molecular, cytogenetical and biochemical characterization of synthetic hexaploid triticale involving chromosome 1D. In: Lelley T (ed) Current topics in plant cytogenetics related to plant improvement. Tulln, Austria, pp 364–370

Hohmann U, Krolow KD (1991) Introduction of D-genome chromosomes from *Aegilops squarrosa* L. into tetraploid triticale (AB)(AB)RR (2n = 28). Theor Appl Genet 82:777–783

Hohmann U, Badaeva K, Busch W, Friebe B, Gill BS (1996) Molecular cytogenetic analysis of Agropyron chromatin specifying resistance to barley yellow dwarf virus in wheat. Genome 39:336–347

Hohmann U, Zoller J, Röbbelen G, Herrmann RG, Kazman ME (1998) Characterization of recombinant hexaploid triticale with improved baking quality. In: Juskiw P, Dyson C (eds) Proc 4th Int Triticale Symp, Red Deer, Canada, Vol 1, pp 208–217

Kazman ME, Lelley T (1994) Rapid incorporation of D genome chromosomes into A- and/or B genomes of hexaploid triticale. Plant Breed 113:89–98

Kazman ME, Lelley T (1996) Can bread-making quality be introduced into hexaploid triticale by whole-chromosome manipulation? In: Guedes-Pinto H, Darvey N, Carnide VP (eds) Triticale: today and tomorrow, Kluwer Academic Publ, Dordrecht, pp 141–148

Kazman ME, Weipert D, Röbbelen G (1996) Entwicklung von Backqualität in hexaploidem Triticale durch chromosomale Substitution. In: Triticale – Züchtung, Erzeugung und Verwendung. (Proc Triticale Symp.), Vortr Pflanzenzuecht 34:197–211

Kota RS, Gill KS, Gill BS, Endo TR (1993) A cytogenetically based physical map of chromosome 1B in common wheat. Genome 36:548–554

Lukaszewski AJ (1990) Frequency of 1RS·1AL and 1RS·1BL translocations in United States wheats. Crop Sci 30:1151–1153

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719

Lukaszewski AJ, Curtis CA (1992) Transfer of the *Glu-D1* gene from chromosome 1D of bread wheat to chromosome 1R in hexaploid triticale. Plant Breed 109:203–210

Lukaszewski AJ, Curtis CA (1994) Transfer of the *Glu-D1* gene from chromosome 1D to chromosome 1A in hexaploid triticale. Plant Breed 112:177–182

Lukaszewski AJ, Gustafson JP (1983) Translocations and modifications of chromosomes in triticale × wheat hybrids. Theor Appl Genet 64:239–248

Lukaszewski AJ, Apolinarska B, Gustafson JP (1987) Introduction of D-genome chromosomes from bread wheat into hexaploid triticale with a complete rye genome. Genome 29:425–430

Naranjo T, Roca A, Giraldez R, Goicoechea PG (1988) Chromosome pairing in hybrids of *ph1b* mutant wheat with rye. Genome 30:639–646

Nasuda S, Friebe B, Busch W, Kynast RG, Gill BS (1998) Structural rearrangement in Chromosome 2M of *Aegilops comosa* has prevented the utilization of the Compair and related wheat-Ae. comosa translocations in wheat improvement. Theor Appl Genet 96:780–785

Payne PI, Nightingale MA, Krattiger AF, Holt LM (1987) The relationship between HMW glutenin subunit composition and the bread making quality of British-grown wheat varieties. J Sci Food Agric 40:51–65

Rayburn AL, Gill BS (1986) Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4:102–109

Werner JE, Endo TR, Gill BS (1992) Towards a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89:11307–11311