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Stabilization of tetraploid triticale with chromosomes from *Triticum aestivum* (ABD)(ABD)RR (2n = 28)

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Summary. F_1 hybrids with the genome constitution ABDERR (2n = 6x = 42) or ABDE(AB)RR (2n = 7x = 49), selected from crosses between either an octoploid *Triticum aestivum/Thinopyrum elongatum* amphiploid and tetraploid *Secale cereale* (AABBDDEE × RRRR) or autoallohexaploid triticale [AABBDDEE × (AB)(AB)RRRR], were backcrossed to tetraploid triticale (AB)(AB)RR and selfed for six generations. Thirty-three different tetraploid F_6 progenies were karyotyped using C-banding. The aneuploidy frequency was 6.6% with 4.0% hypoploids and 2.6% hyperploids. Among 71 plants with 28 chromosomes, 53.5% had a stabilized karyotype while 46.5% were unstabilized with at least one homoeologous group segregating for A-, B-, or D-genome chromosomes. The stabilized plants represent 19 different tetraploid karyotypes with six of them not containing any detectable D-genome chromosomes from *T. aestivum* or E-genome chromosome from *Th. elongatum*. Thirteen lines were (ABD)(ABD)RR tetraploids with one-to-three disomic substitutions of D-genome chromosomes for A- or B-genome chromosomes. No disomic substitution of E-genome chromosomes was identified. On average 0.58 D substitutions per line were determined. Of the seven D-genome chromosomes only four, 1D, 2D, 5D, and 7D, were present in their disomic state. In unstabilized karyotypes, chromosomes 3D, 4D, and 6D were present in their monosomic state. Among all 30 viable plants (42.3%), the order of decreasing frequency of D-genome chromosomes was 5D (25.0%), 1D (20.0%), 2D (10.0%), 6D (5.0%), and 3D (1.7%). Plants with 4D and 7D chromosomes were not viable. An increase in the number of D-genome chromosomes in the (ABD) genome is associated with a decrease in viability and fertility. Minor differences in the C-banding of chromosomes in homoeologous groups 1, 5, and 6 indicate the possibility of translocations between A-, B-, D-, and E-genome chromosomes. Evolutionary and breeding aspects of tetraploid triticale with mixed genomes are discussed.

Key words: Genome stabilization – Aneuploidy – C-banding – D-genome chromosomes

Introduction

Tetraploid triticale (× *Triticosecale* Wittmack) is the youngest wheat-rye hybrid compared to the hexaploid and octoploid triticale forms. The first tetraploids synthesized (Krolow 1973) had the genome constitution (AB)(AB)RR (Gustafson and Krolow 1978). At present, about 144 lines with approximately 56 different chromosome constitutions have been published (Lukaszewski et al. 1984, 1987b; Baum and Lelley 1988; Badaev et al. 1992). The (AB) mixed genome composition of tetraploid triticale (AB)(AB)RR offers the possibility of studying the compensating ability of chromosomes, or chromosome combinations, from the diploid wheat genome when present in a plant with a rye genome. Tetraploid triticale can thus provide an interesting model for the study of genome evolution in polyploids.

In breeding new cultivars of triticale, the occurrence of aneuploids results in a reduced fertility and uniformity of the wheat-rye hybrid (Gregory et al. 1986). Aneuploids often differ in their morphology and their spontaneous occurrence, and are mainly caused by meiotic instability. Therefore, breeding for higher meiotic stability could improve uniformity or performance of triticale.

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Tetraploid triticales are believed to be cytologically the most stable amphiploid, as noted by the presence of a low number of aneuploids. Understanding the mechanism that stabilizes the meiosis of a genome in tetraploids can help clarify the interactions between different wheat and rye genomes, chromosomes, or genes.

The agronomic potential of tetraploid triticale is still of limited value mainly because of its 50–60% yielding capacity compared to hexaploid triticale (Krolow and Lukaszewski 1986). Nevertheless, the importance of tetraploid triticale for improving the breeding of hexaploid triticale has been well documented (Krolow and Lukaszewski 1986; Lukaszewski et al. 1987a). Recently, Hohmann and Krolow (1991) reported on tetraploids with chromosomes of *T. tauschii* [DDRR or (ABD)(ABD)RR] which showed improved kernel characteristics and shorter straw but lower fertility compared to the conventional (AB)(AB)RR tetraploids.

A new program was started to introduce D-genome chromosomes from *T. aestivum* and E-genome chromosomes from *Th. elongatum* into (AB)(AB)RR tetraploid triticale (Vos 1983; Krolow et al. 1985; Krolow and Lukaszewski 1986). An octoploid wheat-couchgrass-amphiploid, a hybrid between *T. aestivum* var. Chinese spring × *Th. elongatum* (AABBDDEE, 2n = 8x = 56), was crossed to tetraploid rye or autoallohexaploid triticale (Vos 1983). The resulting sterile or semi-sterile F_1 hybrids were crossed to (AB)(AB)RR tetraploids, without chromosome 5B, to allow chromosome rearrangement by crossing-over.

As proposed by Krolow (1983) this could help to widen the genetic variation and assist in selecting new tetraploids containing a variety of chromosome constitutions [i.e., (ABD)(ABD)RR, (ABE)(ABE)RR and (ABDE)(ABDE)RR]. With respect to the D-genome chromosomes of *T. aestivum* present in tetraploid triticale, only preliminary results, with 15 different stabilized (ABD)(ABD)RR karyotypes, have been described so far (Krolow et al. 1985; Krolow and Lukaszewski 1986; Lehmann et al. 1991; Badaev et al. 1992). The successful incorporated D-genome chromosomes were identified in homoeologous groups 1, 4, 5, and 7. The present paper is the first report on the study of the chromosomal stabilization process and the identification of all D-genome chromosomes from *T. aestivum* present in young tetraploid triticale plants with the genome constitution (ABD)(ABD)RR.

Materials and methods

An octoploid *Agrotriticum* amphiploid (AABBDDEE, 2n = 8x = 56) was obtained by crossing *T. aestivum* var. Chinese Spring (AABBDD) to diploid *Th. elongatum* var. Matsumura Spring (Japan 1956) followed by colchicine treatment of the F_1 hybrid. This amphiploid has good grain quality and reasonable fertility, but long weak stems (Vos 1983). The diploid *Th. elongatum* seeds were kindly supplied by Dr. J.P. Gustafson who obtained them from Dr. E.N. Larter, University of Manitoba, Winnipeg, Canada. To introduce D- and E-genome chromosomes into triticale, the AABBDDEE octoploid was crossed successfully to diploid *Secale cereale* L. var. Petkus (RR), tetraploid *S. cereale* var. Petkus (RRRR), hexaploid triticale (AABBRR), and autoallohexaploid triticale [(AB)(AB)RRRR] by Vos (1983). In this study only the primary hybrids of the crosses between AABBDDEE × RRRR and AABBDDEE × (AB)(AB)RRRR were analyzed. To obtain secondary fertile recombinants these F_1 hybrids were crossed once or twice to (AB)(AB)RR tetraploids. The chromosome constitution of the (AB) wheat genome with chromosomes 1A, 2A, 3B, 4A, 5A, 6A, and 7B was the same for the (AB)(AB)RRRR triticale as well as for one of the (AB)(AB)RR tetraploids. The other (AB)(AB)RR tetraploid that was used for the second cross had a different chromosome constitution with chromosomes 1B, 2A, 3B, 4B, 5A, 6B, and 7A. The chromosomes of the (AB) mixed genome originated from *Triticum turgidum* L.; the rye chromosomes from *S. cereale*. All F_1 hybrids and subsequent generations were self-pollinated. Plants were selected for morphology, fertility, and short straw.

The F_2, F_4 and F_6 progenies were C-banded to determine their chromosome constitution. The identification of the wheat and rye chromosomes was according to the C-banding karyotype published by Lukaszewski and Gustafson (1983) and corrected by Lukaszewski et al. (1987a). The designation of chromosomes follows the nomenclature system in wheat (Gill et al. 1991).

Results

The chromosome number of two different secondary F_1 progenies obtained by crosses between ABDERR or ABDE(AB)RR hybrids and (AB)(AB)RR tetraploids ranged from 2n = 31 to 38. A second cross with a (AB)(AB)RR tetraploid line reduced the variation of the chromosome number from 2n = 27 to 32 with four plants having 28 chromosomes. In the bulked F_2 of the first cross, the chromosome number ranged from 27 + 1 telosome to 29 with the majority of plants (93.1%) having 28 chromosomes. The variation of the chromosome number increased slightly in the F_4 (27–29) and F_6 (27–30) due to the larger number of plants and lines analyzed. In the F_6, 93.4% of the plants had 28 chromosomes; 6.6% of the plants were aneuploid with 4.0% being hypoploids and 2.6% being hyperploids. All plants had a complete rye genome. Aneuploidy was caused by wheat chromosomes or telosomes that were either absent (monosomic 1D, 5A or 7B; monotelosomic 5DL or 7BL), or present in additon to a disomic pair of homologues (monotelosomic 4BL, disomic 4A, monosomic 1B, disomic 1D; monosomic 4A, disomic 4B, disomic 7A, disomic 7B). During the stabilization process plants were selected for agronomic characters and not for their chromosome number.

Of 27 plants with 28 chromosomes in the F_2, only two (7.4%) had a stabilized karyotype. In the F_4, 24

Table 1. Stabilized chromosome constitution in the homoeologous groups of the wheat genome of (AB)(AB)RR tetraploid and (ABD)(ABD)RR tetraploid triticale

Homoeologous group							Type of mixed genome	Plant no.
1	2	3	4	5	6	7		
A*A*	AA	B*B*	AA	AA	A*A*	BB	$A_5B_2D_0$	115[1], 117[1], 119[1], 120[1], 121[1], 126[1], 128[1], 297[1], 314[1], 336
AA	AA	B^+B^*	AA	A^+A^+	AA	BB*	$A_5B_2D_0$	303, 305[1], 310, 311, 313[1]
BB	A*A*	B*B*	BB	AA	AA	AA	$A_4B_3D_0$	323[1]
BB	AA	BB	BB	A*A*	AA	BB	$A_3B_4D_0$	318[1], 319[1]
BB	AA	BB	BB	A*A*	BB	BB	$A_2B_5D_0$	124[1], 315
BB	AA	BB	BB	AA	BB	BB	$A_2B_5D_0$	316, 317
D^+D^+	AA	BB	AA	AA	AA	BB	$A_4B_2D_1$	233
DD	AA	BB	AA	AA	AA	BB	$A_4B_2D_1$	130[1], 234[1], 300[1]
DD	AA	BB	AA	AA	BB	AA	$A_4B_2D_1$	129[1]
DD	BB	AA	AA	AA	AA	BB	$A_4B_2D_1$	333[1]
D*D*	AA	BB	BB	AA	BB	AA	$A_3B_3D_1$	225
BB	DD	BB	BB	AA	BB	AA	$A_2B_4D_1$	295
AA	AA	BB	AA	DD	AA	BB	$A_4B_2D_1$	111[1]
BB	AA	BB	AA	DD	AA	BB	$A_3B_3D_1$	288
BB	AA	BB	AA	DD	BB	BB	$A_2B_4D_1$	219
DD	DD	BB	BB	AA	BB	AA	$A_2B_3D_2$	291
DD	AA	BB	BB	DD	BB	AA	$A_2B_3D_2$	218[1], 201[1]
DD	AA	BB	BB	AA	BB	DD	$A_2B_3D_2$	229[1]
DD	DD	BB	BB	DD	BB	AA	$A_1B_3D_3$	293[1]

[1] Plants are sublethal
* Some plants with chromosomes of modified C-banding pattern
** Unstabilized karyotype
$^+$ Translocation involving D-genome chromosome arms
1A* 1AL1.3 is located at 0.44, additional band 1AL1.31 at 0.61
1D* Additional terminal bands at 1DS1.41 and 1DL1.61, absent bands 1DL1.3 and 1DL1.5, banding pattern similar to chromosome 3D but substituting for homoeologous group 1
2A* 28, del 2A (S1.5:0.11), terminal deletion with breakpoint in 2AS1.5:0.11 at 0.89, arm ratio similar to chromosome 2D
3B* Banding pattern identical to chromosome 3B of cv. Chinese Spring
5A* Probably Ti5AS.5BL1.1–L1.5:5AL–ter, intercalary translocation of 5BL1.1–L2.1 replacing 5AL1.1–L1.5; 5AS1.1 is located at FL 0.05
6A* Additional bands of 6L1.21 at 0.05, 6L1.22 at 0.18, 6L1.23 at 0.24 and 6L1.24 at 0.35
7B* Probably T7BS.7BL-7AL and absent terminal band 7AL1.7
1A* T1AS.1DL
$1D^+$ T1DS.1AL
$3B^+$ T3BS.3AL
$5A^+$ Probably T5AS.5BL
E? Unidentified chromosome with C-banding pattern unknown in hexaploid wheat but similar to E-genome chromosome (Endo and Gill 1984)

plants (42.1%) had a stabilized genome. Of the 71 plants in the F_6, originating from 33 different progenies, 38 (53.5%) had a stabilized (AB) genome (31,0%) or (ABD) genome (22.5%) with homologous pairs of chromosomes, exclusively. No plants with a pair of E-genome chromosomes were identified. The 22 stabilized (AB)(AB)RR lines were assigned to four different chromosome constitutions representing six different karyotypes. The number of A- and B-genome chromosome pairs ranged from two to five (A_2B_5, A_3B_4, A_4B_3, and A_5B_2), respectively (Table 1). The six stabilized (ABD)-(ABD)RR tetraploids were attributed to 13 different karyotypes (Table 1), with the number of D-genome chromosomes or chromosome arms ranging from one ($A_4B_2D_1$) to three pairs ($A_1B_3D_3$). Only four D-genome chromosomes of *T. aestivum*, 1D, 2D, 5D, and 7D, could be substituted for by A- and (or) B-genome chromosomes at least once (Fig. 1). Ten of the thirteen (ABD) karyotypes had one pair of D-genome chromosomes, with either 1D, 2D, and 5D being present (Table 1). All double substitutions had a 1D(1A) substitution in common and, in addition, a pair of either 2D, 5D, or 7D. Thirty-three plants (46.5%) were unstabilized with at least one homoeologous group segregating for A-, B-, and (or) D-genome chromosomes (Table 2).

Two centric-break-fusion translocations, T1DS.1AL and T5AS.5BL, were identified in the stabilized lines. In unstabilized lines two more translocations, T1AS.1DL

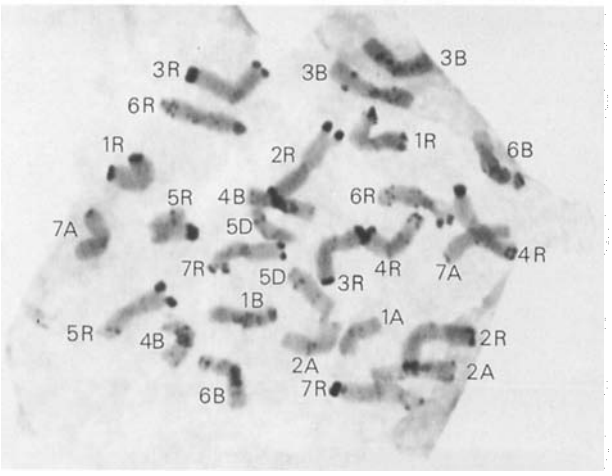

Fig. 1. C-banded mitotic chromosomes of (ABD)(ABD)RR tetraploid triticale with a constitution monosomic for chromosomes 1A and 1B, disomic for chromosomes 2A, 3B, 4B, 5D, 6B, 7A, and with a complete rye genome

and T3AS.3BL, were detected. In 22.5% of (AB)(AB)RR and (ABD)(ABD)RR tetraploids variation was observed in the banding pattern of A-, B-, and D-genome chromosomes indicating the presence of additional structural rearrangements. In the rye genome only minor variation in the amount of heterochromatin was observed involving terminal deletion of heterochromatin on chromosome arms 1RS, 3RS, and 4RL.

Two chromosomes among the 76 plants in the F_6 had a C-banding pattern different from any wheat chromosome. One of these substituted for a chromosome in homoeologous group 2; the other substituted for a chromosome in homoeologous group 5. Both show a C-banding pattern that is similar to the *Th. elongatum* chromosomes designated A and B by Endo and Gill (1984). However, there was no clear evidence for the presence of *Th. elongatum* chromosomes or chromatin in viable tetraploid triticale.

The frequencies of individual disomic D-genome chromosomes were differrent with respect to the seven homoeologous groups (Table 3). The order of D-genome chromosomes of decreasing frequency was 1D (31.6%), 5D (15.8%), 2D (7.9%), and 7D (2.6%). Disomic states of chromosomes 3D, 4D, and 6D were not detected among the stabilized karyotypes. The average number of homoeologous pairs of A-, B-, and D-genome chromosomes in stabilized lines was 3.71, 2.71 and 0.58, respectively. The chromosome constitution of the (AB) genome (chromosomes 1A, 2A, 3B, 4A, 5A, 6A, and 7B) of the tetraploid, which was used for crossing to improve stability and fertility, induced a significant selection pressure for chromosomes 3B and 5A ($P<0.05$). The chromosome combinations 2A-3B (89.5%), 4A-7B [73.9% or 100% in (AB)(AB)RR tetraploids with chromosome

Table 2. Unstabilized chromosome constitution in the homoeologous groups of the wheat genome of tetraploid triticale with possible selection of new karyotypes

Homoeologous group							Plant no.
1	2	3	4	5	6	7	
AB	AA	BB	AA	AA	AB	BB	298
AA	AA	DD	AD	AD	DD	BB	113[1]
AA	AB	BD	AA	AA	AA	BB	307
AD	AE?	B*B*	AA	AA	AA	BB	337
AA	AA	B^+D	AB	AD	AA	BB	304[1]
BD	AB	BB	AB	AA	A*A*	BB	125[1]
DD	AA	BB	BB	AD	AA	BB	227[1]
DD	AA	DD	BB	AB	AA	BB	123[1]
BB	AA	BB	BB	AD	BB	BB	286
BB	AA	BB	AB	BD	BB	BB	215
BB	AA	BB	BB	AD	AB	AA	320
AB	AA	BB	BB	AD	AB	BB	285
BB	AA	BB	BB	AD	BB	AA	243
AB	AA	BB	BB	DD	BB	AA	287
AB	AB	BD	AB	AA	BB	AA	306[1]
DD	AA	BB	AB	AA	AA	AA	214[1]
DD	AA	BB	BB	DE?	BB	AA	216[1]
BD	AA	BB	BB	BD	BB	AA	217[1]
BD	AA	BB	BB	AB	BB	AA	223[1]
BD	AA	BB	AA	AA	AA	AA	131[1]
BD	AA	BB	AA	DD	BB	AA	211[1]
A^*A^+	AA	BB	AA	AA	DD	AA	231
BB	AA	BB	AD	AA	BB	DD	228[1]
A*B	AA	BB	AA	AA	BD	AB	232
AA	AA	BB	BB	AD	BB	BB	294
AA	AA	BB^+	BB	AB	BB	AA	296
BB	AA	BB	BB	AD	AB	AA	242
AA	AA	BB	BB	BD	BD	AA	222[1]

For legend see Table 1

5A], 4A-5A (63.2%), 5A-7B (65.8%), as well as the 4A-5A-7B combination (57.9%), were observed very frequently. Among all plants, based on the chromosome constitution of the (AB) mixed genome, D(A) substitutions in homoeologous groups 1, 2, 4, 5, and 6 were found to be more frequent (9.3%) than B(D) substitutions in homoeologous groups 3 and 7 (1.1%).

Among the 33 lines with an unstabilized (AB) or (ABD) genome, chromosomal segregation was found for A- and B-genome chromosomes (in homoeologous groups 1, 2, 4, 5, 6, and 7), A- and D-genome chromosomes (in homoeologous groups 1, 4, and 5), and (or) B- and D-genome chromosomes (in homoeologous groups 1, 3, 5, and 6). The most unstable group was homoeologous group 5 followed in order of increasing stability by groups 1, 4, 6, 2, 3, and 7. On average, heterologous chromosome pairs involving A-genome chromosomes (AB and AD pairs) were more frequent than chromosome pairs involving no A-genome chromosomes (BD pairs).

In F_6 progeny 57.7% of the plants with 28 chromosomes were sublethal and died prior to heading. Of the 49

Table 3. Frequency (%) and mean number of homoeologous and heterologous chromosome pairs in stabilized and unstabilized tetraploid triticale (2n=28) in the F_6

Homoeologous group	Stabilized triticale, chromosome pairs (%)				Unstabilized triticale, chromosome pairs (%)									
	AA	BB	DD	EE	AA	BB	DD	EE	AB	AD	AE?	BD	BE	DE?
1	42.1	26.3	31.6	–	27.3	18.2	15.2	–	15.2	6.1	–	18.2	–	–
2	89.5	2.6	7.9	–	81.8	–	6.1	–	9.1	–	3.1	–	–	–
3	2.6	94.4	–	–	84.9	6.1	–	–	–	–	–	9.1	–	–
4	63.2	36.8	–	–	33.3	45.5	–	–	15.2	6.1	–	–	–	–
5	84.2	–	15.8	–	36.4	–	6.1	–	9.1	36.4	–	9.1	–	3.1
6	65.8	34.2	–	–	36.4	39.4	6.1	–	12.1	–	–	6.1	–	–
7	23.6	73.7	2.6	–	45.5	48.5	3.0	–	3.0	–	–	–	–	–
Mean pairs	3.71	2.71	0.58	–	2.61	2.36	0.42	–	0.63	0.48	0.03	0.42	–	0.03

Table 4. Frequency (%) and mean number of homoeologous and heterologous chromosome pairs in viable stabilized and unstabilized tetraploid triticale (2n=28) in the F_6

Homoeologous group	Stabilized triticale, chromosome pairs (%)				Unstabilized triticale, chromosome pairs (%)									
	AA	BB	DD	EE	AA	BB	DD	EE	AB	AD	AE?	BD	BE	DE?
1	23.1	46.2	30.8	–	23.5	29.4	11.8	–	17.6	11.8	–	5.9	–	–
2	84.6	–	15.4	–	82.4	–	5.9	–	5.9	–	5.9	–	–	–
3	–	100.0	–	–	–	88.2	–	–	–	–	–	11.8	–	–
4	53.8	46.2	–	–	41.2	52.9	–	–	5.9	–	–	–	–	–
5	84.6	–	15.4	–	35.3	–	5.9	–	5.9	47.1	–	5.9	–	–
6	46.2	53.8	–	–	23.5	–	5.9	–	23.5	–	–	5.9	–	–
7	23.1	76.9	–	–	41.2	52.9	–	–	5.9	–	–	–	–	–
Mean pairs	3.15	3.23	0.62	–	2.47	2.65	0.29	–	0.65	0.59	0.06	0.29	–	–

Table 5. Spike morphology and fertility of stabilized and unstabilized tetraploid triticale (2n=28) in the F_6

Triticale	No. lines	Spike length			Spikelets/spike			Seeds/spike			Seeds/spikelet		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
(ABD)(ABD)RR													
Stabilized	7	11.9	10	13	31.6	26	40	18.9	1	41	0.59	0.03	1.38
Unstabilized	17	11.6	7	14	33.0	20	43	19.4	0	53	0.59	0.00	2.00
(AB)(AB)RR													
Stabilized	6	11.2	9	12	32.0	27	38	14.0	0	28	0.46	0.00	1.04
Unstabilized	1	11.0	11	11	23.0	23	23	18.0	18	18	0.78	0.78	0.78
Parents	2	13.5	13	14	39.0	36	42	16.5	4	29	0.37	0.11	0.63

plants with D-genome chromosomes, 26 plants (53.1%) did not survive. On average, the number of A-genome chromosomes (3.15 pairs) decreased, and the number of B-genome chromosomes (3.23 pairs) increased, among viable tetraploids (Table 4) from all plants analyzed in the F_6 (Table 3). The frequency of plants carrying homologous pairs of chromosome 1A and (or) 6A, as well as the heterologous chromosome pair 1B/1D, is drastically reduced. In the karyotype of viable plants the satellited wheat chromosomes of the B-genome, 1B and 6B, occur preferentially. Plants with chromosomes 4D and (or) 7D were not viable. Among all 30 viable plants (42.3%) the order of increasing frequency of D-genome chromosomes was 3D (1.7%), 6D (5.0%), 2D (10.0%), 1D (20.0%), and 5D (25.0%) with 5D being the most substituted D-genome chromosome of *T. aestivum* in te-

Fig. 2. Spike morphology of (AB)(AB)RR tetraploid triticale and of three different (ABD)(ABD)RR tetraploid triticale lines representing (from left to right) single 1D(1A), 5D(5A) and 6D(6A) substitutions

traploid (ABD)(ABD)RR triticale. An increase in the number of D-genome chromosomes in the (ABD) genome is associated with a decrease in viability, and significantly reduced fertility.

Single disomic substitutions set 0.67 seeds per spikelet on average. The double disomic lines set only 0.06 seeds per spikelet. Plants with a 5D(5A) substitution had the best fertility (0.71 seeds per spikelet) followed by plants with a 2D(2A) or a 1D(1A) substitution. Unstabilized (ABD)(ABD)RR are not reduced in fertility (Table 5). In contrast, plants heterologous for chromosomes in homoeologous groups 2, 3, 5, and 6 had improved seed set compared to plants with homologous pairs of chromosomes in those groups. The most fertile line was found among unstabilized (ABD)(ABD)RR tetraploids with the genome constitution 1BB, 2AA, 3BB, 4BB, 5AD, 6BB and 7BB and a seed set of two seeds per spikelet. This indicates the possibility for the further selection of improved lines. Lines with B-genome chromosomes in homoeologous groups 1, 4, an 7 have the best fertility. The fertiliy of (ABD)(ABD)RR triticale is better than estimated for (AB)(AB)RR tetraploids without D-genome chromosomes. The new (ABD)(ABD)RR triticale that were selected during genome stabilization show differences in spike morphology (Fig. 2), have a shorter straw (76.3 cm) and improved kernel characteristics compared to the (AB)(AB)RR tetraploids.

Discussion

Evolution

The evolution of tetraploid triticale can be described as a developing hybrid swarm of different chromosome constitutions as proposed by Gustafson (1976) for hexaploid triticale. In the evolution of polyploid species, Zohary and Feldman (1962) suggested the existence of a pivotal genome that provided stability and fertility, and a differential genome that can segregate and recombine. In tetraploid (AB)(AB)RR triticale (Lukaszewski et al. 1987b) and (ABD)(ABD)RR triticale (Hohmann and Krolow 1991), the rye genome represents the pivotal genome and allows the wheat genome to recombine. This can occur both by interchromosomal recombination, producing new chromosome constitutions, and by the exchange of segments between homoeologous chromosomes. Novel arm combinations, originating from centric-break-fusion translocation, should appear in those progenies where there was little pairing between the differential genomes so leading to univalents and their misdivision during meiosis.

The substitution pattern and the frequency with which individual D- and E-genome chromosomes replace their homoeologous A- and/or B-genome chromosomes in tetraploid triticale is determined mainly by the ability of those chromosomes to compensate for the A- and B-genome chromosomes and by the chromosome constitution of the parents involved in the crossing scheme.

Self-pollination in progenies of primary hybrids from crosses between AABBDDEE × RRRR and AABBDDEE × (AB)(AB)RRRR leads to the random segregation of the four haploid (ABDE) genomes. Theoretically, 16,384 (4^7) different chromosome combinations can be expected. To obtain secondary fertile recombinants these F_1 hybrids were crossed once or twice to (AB)(AB)RR tetraploids. The use of the same (AB) wheat component (i.e., 1A, 2A, 3B, 4A, 5A, 6A, and 7B) helped to stabilize and fix certain chromosomes in the mixed genome. The second cross, with a tetraploid having a different constitution of chromosomes, i.e., 1B, 2A, 3B, 4B, 5A, 6B, and 7A, is expected theoretically to induce heterologous (AB) chromosome pairs in groups 1, 4, 6, and 7 and so increase the probability of introducing B-genome chromosomes and of maintaining whole D- and E-genome chromosomes in those groups. Interestingly, this study has shown that, with the exception of chromosome 1D, there was no preferential substitution of D- and E-genome chromosomes in these homoeologous groups. Moreover, the presence of heterologous pairs of chromosomes may increase instability. Most of the chromosomes or telosomes that were responsible for aneuploidy originate from groups 1 (1B, 1D), 4 (4A, 4BL), and 7 (7A, 7B, 7BL). Univalent chromosomes facilitated the occurrence of telosomes and, therefore, the formation of new centric-break-fusion translocations. The induced homologous chromosome pairs in groups 2 (2A), 3 (3B) and 5 (5A) favoured the stabilization of these groups. This may have had an impact on the directed synthesis of tetraploid triticale with specific chromosome combinations useful for chromosome engineering.

Genome stabilization

The aneuploidy frequency in the F_6 progenies of (ABD)-(ABD)RR tetraploids with D-genome chromosomes from *T. aestivum* was 6.6%, lower than the frequency of 10.7% reported for F_5 progenies of (ABD)(ABD)RR tetraploids with D-genome chromosomes from *T. tauschii*. The high number of 28-chromosome plants in the range characteristic for conventional (AB)(AB)RR tetraploids (Krolow 1974; Hohmann 1985) favored the earlier conclusioin of Lukaszewski et al. (1987b, c) that selection pressure against aneuploid gametes in tetraploid triticale must be stronger than in hexaploid or octoploid triticale. On the other hand, the frequency of 46.5% of plants still segregating for A-, B-, and (or) D-genome chromosomes in the F_6 indicates a slow stabilization process. The continuous process of genome stabilization was proven by 7.4%, 42.1%, and 53.5% of plants having 14 homologous chromosome pairs and stabilized karyotypes in the F_2, F_4, and F_6, respectively. The frequency of stabilized karyotypes was comparable to the frequency that was found in the F_5 of (ABD)(ABD)RR tetraploids with D-genome chromosomes from *T. tauschii* (Hohmann and Krolow 1991).

The average number of chromosome pairs of the A-genome (3.71 pairs), B-genome (2.71 pairs), and D-genome (0.58) was not in an agreement with the assumption of a random distribution and similar compensating ability. The D-genome chromosomes showed a tendency to be substituted for by A-genome chromosomes in homoeologous groups 1, 2, 4, 5, and 6 rather than for B-genome chromosomes in groups 3 and 7. It is not clear at the moment if this depends primarily on the chromosome constitution of the wheat genome of the (AB)(AB)RR tetraploid triticale that was used for backcrossing, or on the compensating ability of the individual chromosomes. In progenies of (ABD)(ABD)RR tetraploids with *T. tauschii* two A-genome chromosomes, 2A and 6A, could be replaced by D-genome chromosomes (Hohmann and Krolow 1991). Two lines of pure (BD)(BD)RR tetraploids without any A-genome chromosomes were identified. In the present study the selection of plants for fertility, short straw, and good kernel characteristics demonstrated that B(A) and D(A) substitutions occurred preferentially during the stabilization process. Results on hexaploid triticale indicate that D(A) substitutions occur more frequently than D(B) substitutions (Krolow and Lukaszewski 1986; Lukaszewski et al. 1987a). The 6D(6A) substitution, in particular, was characterized by improved seed set (Lukaszewski et al. 1987a) and appeared in breeding programs with noticeable frequencies (Lukaszewski and Gustafson 1987). On the other hand, Hohmann (1988) reported only D(A) substitutions in combination with D(B) substitutions resulting in (AD)(AD)(BD)(BD)RR hexaploids.

Translocations and chromosome combinations

Translocations between non-homoeologues tend to limit the spectrum of possible chromosome combinations in tetraploid triticale (Lukaszewski et al. 1984; Hohmann and Krolow 1991). For example, in homoeologous groups 4 and 7 certain chromosome combinations appeared frequently and others were absent. In (ABD)(ABD)RR tetraploids the D-genome chromosomes 4D and 7D could be substituted successfully only in the presence of an additional 5D(5A) or 5D(5B) substitution (Hohmann and Krolow 1991; Badaev et al. 1992). For a line to be fertile, usually either all or none of these three chromosomes have to be substituted.

The occurrence of a cyclical translocation involving chromosomes 4A, 5A, and 7B (Gill and Chen 1987; Naranjo et al. 1987; Liu et al. 1992) in hexaploid wheat should favor the selection of karyotypes containing the chromosome combinations 4A, 5A, and 7B in tetraploid triticale. In the present study, all (AB)(AB)RR tetraploids without D-genome chromosomes and chromosome 5A have chromosomes 4A and 7B. Of all karyotypes analyzed in this study 57.9% had the 4A-5A-7B combination. Obviously, the common 5D(5A) substitution reduced their frequencies. Chromosome 5D of *T. aestivum* should have a good compensating ability in tetraploid triticale. The occurrence of a high frequency of fertile plants segregating for chromosome 5D/5A in this study, or for 5D/5B (Hohmann and Krolow 1991), indicates that the monosomic state of chromosome 5D may improve species fitness. Plants with heterologous chromosome pairs have a qualitatively larger genetic variation. These plants may have a better vitality or adaptability during the process of genome stabilization in tetraploid triticale. The presence or absence of satellites and the size of the chromosomes involved could also affect the frequency of meiotic disturbances and possibly result in a natural selection pressure (Gustafson 1976). In fact, among the (ABD)(ABD)RR tetraploids analyzed here and earlier (Krolow et al. 1985; Krolow and Lukaszewski 1986; Hohmann and Krolow 1991) the small SAT-chromosome 5D appeared to be the most frequent one. Selection during the stabilization process favored the 1D(1B) and the 5D(5A) or 5D(5B) substitution to replace chromosome 1B and introduce chromosome 5D into tetraploid triticale (Hohmann and Krolow 1991).

Obviously, in tetraploid triticale the compensating centric-break-fusion translocations T5AS.5RL (Lukaszewski et al. 1984), T3AL.3BL, T5AS.5BL (Lukaszewski et al. 1987b), T5DS.5AL (Lukaszewski and Gustafson 1987), and the new T3AS.3BL, T1DS.1AL and T1AS.1DL translocations identified in this study, occur mainly between homoeologous chromosomes. The T1DS.1AL translocation in tetraploid triticale reported

here was found to be present in hexaploid triticale as well (Lukaszewski et al. 1987a). Chromosome translocations between A- and D-genome chromosomes which compensate in tetraploid triticale should also have good compensating ability in hexaploid triticale.

Intrachromosomal recombination

One requirement for the successful transfer of smaller DNA segments is meiotic recombination between homoeologues. Therefore, the (AB)(AB)RR tetraploids that were used for crossing to produce fertile secondary (ABD)(ABD)RR tetraploids did not carry chromosome 5B. Chromosome 5B was the only chromosome of the (AB) component that was not identified in stabilized karyotypes in the F_6.

Nevertheless, the induction of homoeologous pairing caused by the suppression or absence of the *ph* locus located on chromosome 5B is not necessary in the tetraploids. Chromosome pairing between homoeologues is a common phenomenon in tetraploid triticale. The high pairing frequency was explained by translocations that have accumulated during line development (Lukaszewski et al. 1987b). On the other hand, pairing of A- and B-genome chromosomes (Baum and Lelley 1988) as well as of A- and D- or B- and D-genome chromosomes (Hohmann and Krolow 1991) has been reported in primary F_1 hybrids with the genome constitution ABRR or D(AB)RR, respectively. In advanced lines of tetraploids the selection pressure may have been useful for retaining chromosomes or genes promoting synapsis (Lukazewski et al. 1987b) and inducing chromosomal stability and recombination in tetraploids with low aneuploid frequencies. The chromosome constitution in tetraploids with low aneuploid frequencies. The chromosome constitution of (ABD)(ABD)RR tetraploids indicates that there is a tendency for the presence of chromosomes that carry pairing-promoting genes i.e., chromosomes 2A, 6A, 6B (Dvorak and McGuire 1981; Kota and Dvorak 1986), 3B (Sears 1954), 5A and 5D (Feldman 1966; Viegas et al. 1980), and a tendency for the absence of chromosomes that carry pairing-suppression genes i.e., chromosomes 4D (Driscoll 1973), 7A (Miller and Reader 1985), 7D (Dvorak and McGuire 1981), 4E and 7E (Dvorak 1987). The latter may be helpful for retaining alien chromosomes or chromosome segments in the wheat mixed genome by suppressing homologous pairing and (or) inducing pairing and recombination between homoeologous chromosomes.

Conclusion

The selection of stabilized tetraploid is a time- and labor-intensive process since the high frequency of fertile plants segregating for A-, B- and (or) D-genome chromosomes could help to maintain these unstabilized chromosome constitutions in a population (Lukaszewski et al. 1987c; Hohmann and Krolow 1991). Double monosomic plants have a qualitatively larger genetic variaton and, therefore, these plants may have a better adaptability or vitality during the process of genome stabilization compared to plants with 14 pairs of homologous chromosomes. This may have significance for the breeding of stabilized tetraploid triticale.

Using in-situ hybridization (ISH) in combination with genome-specific and dispersed sequences, or genomic ISH (GISH), will serve to detect smaller translocations that are probably present in the analyzed, so called "stabilized", karyotypes. In tetraploid triticale the expression of these segments can be studied in the presence of a rye genome and the absence of another wheat homoeologue.

In summary, for the transfer of alien DNA segments from *Th. elongatum* into tetraploid triticale the suppression of homoeologous pairing may not have been very successful. The detection of centric-break-fusion translocations between D-genome chromosome arms and arms of the A- and B-genome chromosomes have been shown to be more effective in retaining larger segments of alien chromatin in tetraploid triticale. They offer the possibility, for example, of transferring segments of D-genome chromosomes from tetraploid triticale to hexaploid triticale or tetraploid wheat.

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