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Characterization of rust-resistant wheat-*Agropyron intermedium* derivatives by C-banding, in situ hybridization and isozyme analysis

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Summary. Chromosome constitutions of three wheat-*Agropyron intermedium* derivatives were identified by C-banding analysis, in situ hybridization using biotin-labeled genomic *Ag. intermedium* DNA as a probe and isozyme analysis. Lines W44 and W52 were identified as 7Ai-2(7D) and 7Ai-2(7A) chromosome substitution lines carrying the same chromosome pair of *Ag. intermedium*. The alien chromosome was found to be homoeologous to group 7 based on C-banding, meiotic pairing and isozyme analyses. Line W49 was identified as a wheat-*Ag. intermedium* chromosome translocation line. The breakpoint of the T2AS · 2AL-7Ai-2L translocation is located in the long arm at a fraction length of 0.62, and the transferred *Ag. intermedium* segment has a size of about 2.4 μm. Lines W44 and W52 expressed *Ag. intermedium* genes for resistance to leaf rust, stripe rust and stem rust, but only leaf rust resistance was expressed in W49. The results show that the leaf rust resistance gene(s), designated Lr38, is located in the distal half of the long arm of chromosome 7Ai-2, whereas the genes for resistance to stem rust and stripe rust are located either in the short arm or in the proximal region of the long arm of this chromosome.

Key words: Wheat-*Agropyron intermedium* derivatives – Stem rust – Leaf rust – Stripe rust – C-banding – In situ hybridization – Isozyme analysis

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Introduction

Leaf rust, stripe rust and stem rust caused by the pathogens *Puccinia recondita* Rob. ex Desm. f. sp. *tritici, Puccinia striiformis* West. f. sp. *tritici* and *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn., respectively, are serious diseases of cultivated wheat, *Triticum aestivum* L. em Thell (2n = 6x = 42, genomically AABBDD). Wild species of the genus *Agropyron* are known to be important sources of resistance to rust diseases (Knott 1989) and have been used in breeding programs aimed at transferring resistance into this crop.

Knott (1961) and Sharma and Knott (1966), following a method developed by Sears (1956) using X-ray treatment to induce wheat-alien chromosome translocations, reported the successful transfer of stem rust and leaf rust resistances from *Agropyron elongatum* (Host) P. B. to hexaploid wheat. Multiple transfers of leaf rust resistance derived from *Ag. elongatum* using induced homoeologous chromosome pairing were reported by Sears (1973). A second important source of rust resistance is the hexaploid species *Agropyron intermedium* (Host) P.B. (2n = 6x = 42), genomically E_1E_2X, where the E_1- and E_2-genomes are related to the E-genome of *Ag. elongatum* and the *X*-genome is of an unknown origin (Dvořak 1981 a, b; Dewey 1984; Endo and Gill 1984).

Cauderon (Cauderon 1966; Cauderon et al. 1973; Cauderon and Rhind 1976) produced a series of six wheat-*Ag. intermedium* chromosome addition lines in the cv 'Vilmorin 27' and showed that three of the added *Ag. intermedium* chromosomes carried genes for resistance to each of the three diseases. Other sets of wheat-*Ag. intermedium* chromosome addition lines carrying resistance to leaf rust and stripe rust have been produced by Sinigovets (1976) and Zeller (Hsam and Zeller 1982; Bartos and Zeller, unpublished results).

Wienhues (1960) reported the transfer of a chromosome pair derived from *Ag. intermedium* to hexaploid wheat cv 'Heine IV', thus conditioning resistance to leaf rust, stripe rust and stem rust. By crossing the chromosome addition line with the monosomic set of 'Chinese Spring' and using X-ray treatment she selected rust-resistant wheat-*Ag. intermedium* chromosome substitution and translocation lines (Wienhues 1966, 1967, 1971, 1973, 1979). In the present study we describe the resistance pattern and chromosome constitutions of three advanced wheat-*Ag. intermedium* lines derived from material of Dr. A. Wienhues.

C-banding analysis, which enables the identification of all 21 chromosome pairs and most chromosome arms of hexaploid wheat (Gill et al. 1991), and in situ hybridization using biotin-labeled total genomic *Ag. intermedium* DNA as a probe were used to identify *Ag. intermedium* chromatin. Both techniques are known to be highly efficient in detecting alien chromatin in wheat (Rayburn and Gill 1985; Lapitan et al. 1986; Le et al. 1989; Anamthawat-Jonsson et al. 1990; Mukai and Gill 1991; Mukai et al. in press; Friebe et al. 1991 a, b). Isozyme analysis was used to confirm the homoeologous relationships of the critical *Ag. intermedium* chromosome.

Material and methods

Plant material

Three wheat-*Ag. intermedium* derivatives designated W44, W52 and W49 (2n = 42) kindly supplied by Dr. Nitzche, Max Planck Institut für Züchtungsforschung, Köln-Vogelsang, Germany were analyzed in the present study. All three lines were selected by A. Wienhues in a program aimed at transferring resistances to leaf rust, stripe rust and stem rust from *Ag. intermedium* to hexaploid wheat. Lines W44, W52 and W49 were derived from a partial amphiploid *T. aestivum* – *Ag. intermedium* (2n = 8x = 56) carrying seven *Ag. intermedium* chromosomes added to the chromosome complement of hexaploid wheat. By backcrossing this amphiploid with hexaploid wheat cv 'Heine IV', she obtained *T. aestivum* – *Ag. intermedium* chromosome addition lines in a 'Heine IV' background. These lines were then either crossed with the monosomics of hexaploid wheat cv 'Chinese Spring' or, after X-ray treatment, backcrossed with 'Heine IV' in order to produce rust-resistant chromosome substitution and translocation lines, respectively. The detailed production and resistance screening of this material has been described earlier (Wienhues 1960, 1966, 1967, 1971, 1973, 1979). The recipient wheat cv 'Heine IV' and the wheat-*Ag. intermedium* chromosome addition line, T2 (2n = 44), were included in the analysis.

Cytogenetic analysis

Chromosome identification was carried out either by the conventional Feulgen staining procedure or according to the C-banding technique described by Gill et al. (1991). The method of Mukai et al. (1991) was used for in situ hybridization, but with the following modifications. Total genomic DNA's were extracted from *Ag. intermedium* (accession 75, kindly provided by Y. Cauderon, France) and *T. aestivum* cv 'Chinese Spring'. 500 ng of *Ag. intermedium* genomic DNA was labeled with biotin-16-dUTP by the random primer method (Boehringer, Mannheim). The hybridization mixture consisted of (final concentrations) 50% formamide, 2 × SSC, 10% dextran sulfate, salmon sperm DNA at 1.5 mg/ml, biotin-labeled *Ag. intermedium* genomic DNA at 2.5 µg/ml and unlabeled wheat genomic DNA at 50 µg/ml. Hybridization reactions were carried out at 42 °C for 16 h following post-hybridization washes at 42 °C instead of 37 °C. Chromosome measurements were carried out to determine translocation breakpoints. Positions of translocation breakpoints were calculated as fractions of the total chromosome arm lengths from the centromere (fraction length).

Isozyme analysis

Isozyme analysis was carried out after separation in ultra-thin isoelectric focusing (IEF) gels. Three enzyme systems, α-amylase-2 (α-AMY-2), peroxidase-4 (PER-4) and endopeptidase-1 (EP-1), were studied. The genes *α-Amy-2* and *Ep-1* are located on the long arms of homoeologous group 7 chromosomes; *Per-4* is located on the short arms of group 7 homoeologous chromosomes (McIntosh 1988). Protocols for α-AMY-2 and PER-4 were those described by Forster et al. (1987), whereas EP-1 was extracted and stained as described by Koebner et al. (1988) except that enzyme activity was detected directly in the IEF gel.

Resistance analysis

Rust resistance tests were carried out on seedlings in the greenhouse with leaf rust races 14, 14SaBa, 61, 53SaBa, 77SaBa (SaBa designates virulence to cv 'Salzmünder Bartweizen') and stem rust races 21 and 11 in Czechoslovakia and leaf rust race 104-2,3,6,(7), stem rust race 34-1,2,3,6,7,9 and stripe rust race 108 E141A$^+$ in Australia.

Results

Cytogenetic analysis

C-banded mitotic metaphases of lines W44, W52 and W49 are shown in Fig. 1 and detailed C-banded karyotypes of W44 and W49 are presented in Figs. 2 and 3, respectively. All lines show an euploid chromosome number of 2n = 42, indicating either chromosome substitution or translocation.

In lines W44 and W52 one chromosome pair of wheat is missing and is replaced by a complete chromosome pair of *Ag. intermedium*. This chromosome pair is identical in morphology and in C-banding pattern in both lines (Figs. 2, 4 a, b). The missing wheat chromosome pair of line W44 was identified by C-banding analysis as chromosome 7D, whereas line W52 lacked chromosome pair 7A. Since lines W44 and W52 are fertile, the compensation of the *Ag. intermedium* chromosome for wheat chromosomes 7A and 7D indicates that the alien chromosome is homoeologous to group 7. Thus, the *Ag. intermedium* chromosome was designated 7Ai-2, and lines W44 and W52 can be described as 7Ai-2(7D) and 7Ai-2(7A) substitution lines, respectively. The maximum chromosome pairing at metaphase I in pollen mother cells of F_1 hybrids between CS double ditelosomic 7D

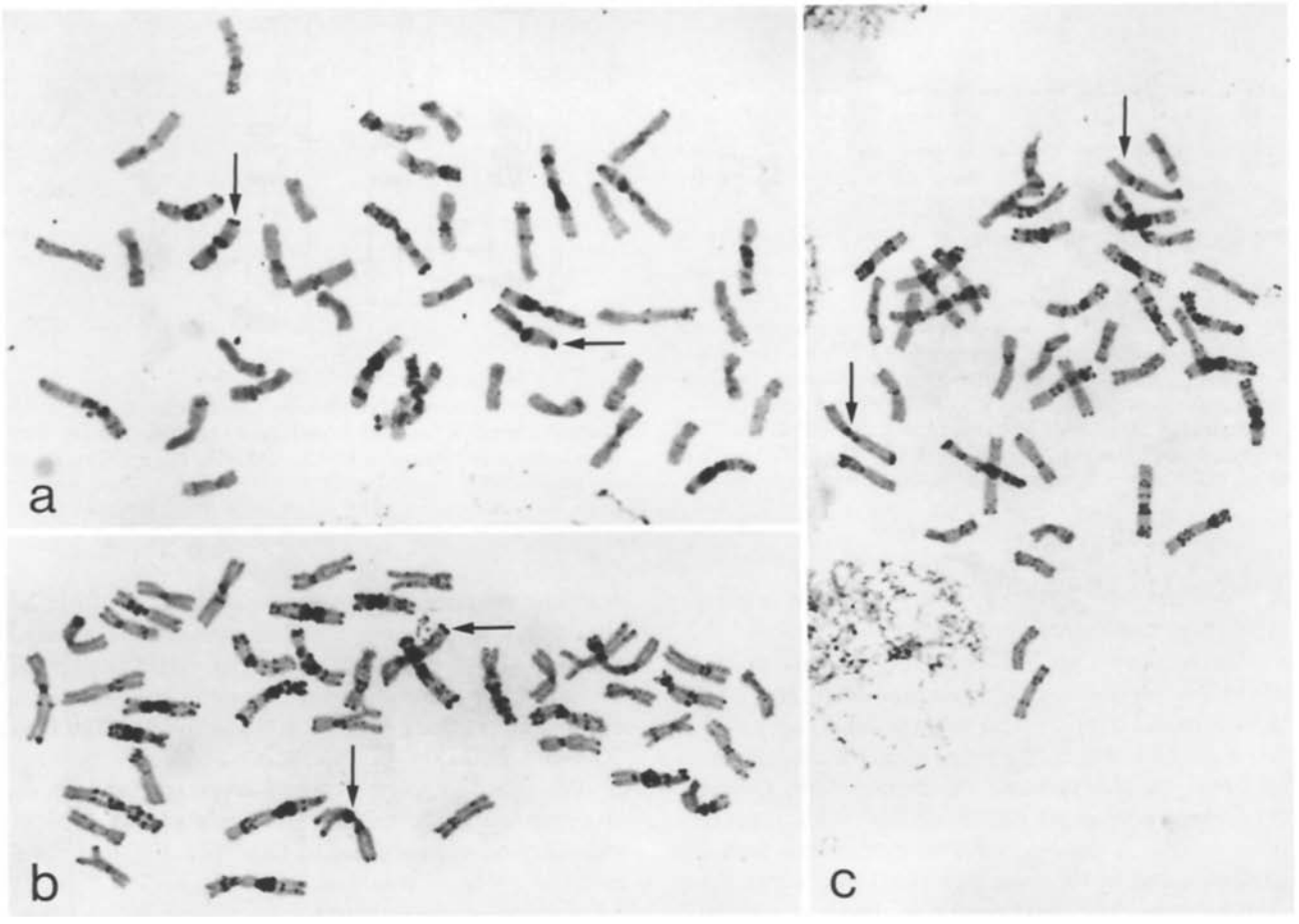

Fig. 1a–c. C-banded mitotic metaphases of wheat-*Ag. intermedium* derivatives W52 (**a**), W44 (**b**) and W49 (**c**) (*arrows* point to the *Ag. intermedium* or wheat-*Ag. intermedium* translocation chromosomes)

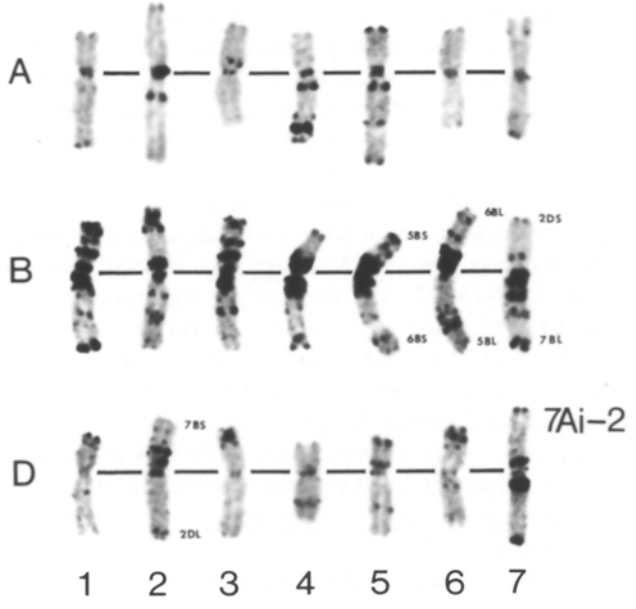

Fig. 2. C-banded karyotype of wheat-*Ag. intermedium* derivative W44

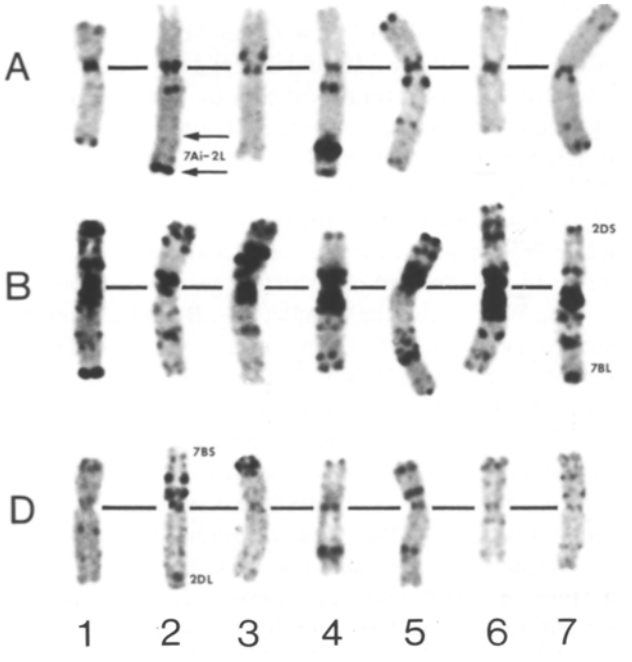

Fig. 3. C-banded karyotype of wheat-*Ag. intermedium* derivative W49

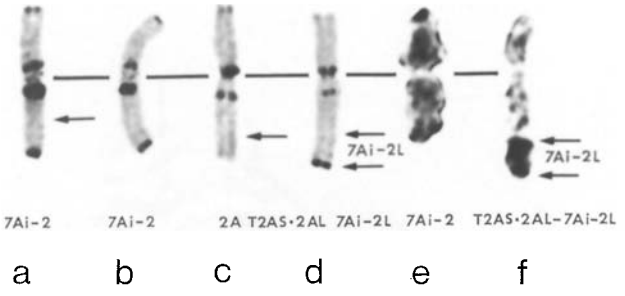

Fig. 4. C-banding and in situ hybridization patterns using biotin-labeled total genomic *Ag. intermedium* DNA as the critical wheat, *Ag. intermedium* and wheat-*Ag. intermedium* translocation chromosomes: **a** 7Ai-2 of W44, C-banding, **b** 7Ai-2 of W52, C-banding, **c** 2A of W44, C-banding, **d** T2AS · 2AL-7Ai-2L of W49, C-banding, **e** 7Ai-2 of W44, ISH, **f** T2AS · 2AL-7Ai-2L of W49, ISH (*arrows* point to the translocation breakpoints)

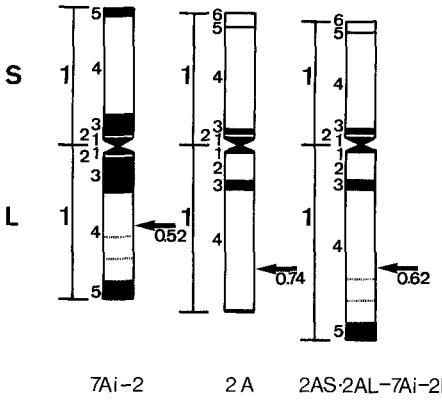

Fig. 5. Comparison of chromosome 2A of wheat, the *Ag. intermedium* chromosome 7Ai-2 and the wheat-*Ag. intermedium* translocation chromosome T2AS · 2AL-7Ai-2L (*arrows* point to the translocation breakpoints)

and W44 was $16''+2IV+1'+t'+t'$, confirming a complete alien chromosome substitution.

Measurements on 20 C-banded 7Ai-2 chromosomes of line W44 were made; the mean total length was 9.5 μm (S: 4.5 μm; L: 5.0 μm) with an arm ratio (L/S) of 1.1. 7Ai-2 can be easily distinguished from the chromosomes of wheat by the presence of characteristic C-bands. Prominent C-bands are present on both sides of the centromere and at the telomeres of both arms, with the bands located in the long arm (L1.3 and L1.5) being slightly larger than those present in the short arm (S1.3 and S1.5) (Figs. 4a, b, 5). Occasionally, two additional very small C-bands were observed in the distal half of the long arm.

It was also evident from C-banding analysis that chromosomes 5B, 6B, 7B and 2D of Line W44 are involved in reciprocal translocations, the breakpoints being located within the centromeric regions (Fig. 2). The resulting translocations can be described as T5BS · 6BS, T5BL · 6BL, T7BS · 2DL and T2DS · 7BL. Only the T7BS · 2DL and T2DS · 7BL translocations are present in line W52 in which chromosomes 5 and 6 of the B-genome are not involved in structural rearrangements.

Line W49 carries the complete A-, B- and D-genome complement of wheat. Chromosomes 7B and 2D are present as T7BS · 2DL and T2DS · 7BL translocations similar to those found in W44 and W52 (Fig. 3). However, line W49 does not carry a complete *Ag. intermedium* chromosome 7Ai-2 but only a segment of this chromosome in the form of a wheat-*Ag. intermedium* chromosome translocation. The wheat chromosome involved in this translocation was identified by C-banding as chromosome 2A. The C-banding pattern of the 2A translocation chromosome differs from that of a normal chromosome 2A with respect to the distal region of the long arm. In W49 the long arm of chromosome 2A shows the characteristic marker C-band of 2AL close to the centromere (L1.3) and, in addition, a prominent telomeric C-band and two very small, distally located C-bands that are similar in size and location to those present in the long arm of the *Ag. intermedium* chromosome 7Ai-2 of lines W44 and W52 (Fig. 4c, d).

Chromosome measurements were carried out on 20 C-banded 2A chromosomes of line W44 and on 20 2A translocation chromosome of line W49. Whereas chromosome 2A has a total length of 9.7 μm (S: 4.3 μm; L: 5.4 μm) and an arm ratio of 1.3, the corresponding values for the 2A translocation chromosome are 10.4 μm (S: 4.0 μm; L: 6.4 μm) and (L/S) 1.6, respectively. Mean lengths of the short and long arms were compared by applying Student's *t*-test. Whereas no statistically significant difference was found to occur between the mean lengths of the short arms of chromosome 2A and the 2A translocation chromosome ($t=1.8$; $df\,38$), the estimated value of $t=-4.0$ for the comparison of the long arms was highly significant (with $P<0.01$ and $df\,38$, significance limits of $t=\pm 2.7$). The wheat-*Ag. intermedium* chromosome consists of the complete short arm of 2A, a large region of the long arm of 2A and a distal segment derived from the long arm of 7Ai-2, and can be described as T2AS · 2AL-7Ai-2L. The breakpoints are located in region L1.4 of 7Ai-2 and in L1.4 of chromosome 2A (Fig. 5), and thus the complete designation of this translocation is T2AS · 2AL1.4::7Ai-2L1.4 (according to the standard nomenclature system of wheat proposed by Gill et al. 1991). However, due to the lack of marker C-bands in region L1.4, it was not possible to identify the exact breakpoints of this translocation by C-banding analysis alone.

In situ hybridization (ISH) using biotin-labeled total genomic *Ag. intermedium* DNA as a probe in combination with unlabeled genomic wheat DNA as a competitor

was used for the localization of the exact breakpoint of the T2AS · 2AL-7Ai-2L translocation, and the ISH patterns of the critical chromosomes are shown in Fig. 4e, f. In W44 only one chromosome pair shows hybridization over the entire lengths of both arms (Fig. 4e), whereas all the other chromosomes of the complement are unlabeled. In W49 only the distal region of the long arm of one chromosome pair shows hybridization with the *Ag. intermedium* DNA probe (Fig. 4f). Measurements on ten ISH-labeled translocation chromosomes of W49 revealed that the labeled region consists of the distal 38% of the long arm of this chromosome. This locates the breakpoint of the wheat-*Ag. intermedium* translocation in region L1.4 of chromosome T2AS · 2AL-7Ai-2L at a fraction length of 0.62 (Fig. 5). Combining the ISH data with the measurements based on C-banded chromosome T2AS · 2AL-7Ai-2L, we were able to calculate the size of the transferred *Ag. intermedium* in the translocation chromosome. The translocated *Ag. intermedium* segment has a size of 2.4 μm (corresponding to 48% of 7Ai-2L). The original breaks occurred in region L1.4 of chromosome 7Ai-2 at a fraction length of 0.52 and in region L1.4 of chromosome 2A at a fraction length of 0.74. The missing distal 2AL segment in the T2AS · 2AL-7Ai-2L translocation has a size of 1.4 μm (corresponding to 26% of 2AL).

In order to verify the homology of the *Ag. intermedium* segment in the T2AS · 2AL-7Ai-2L translocation with the distal region of the long arm of chromosome 7Ai-2, line W44 was crossed with W49, and meiotic pairing was analyzed in C-banded PMC's of the F_1 hybrid. A total of 123 PMC's were analyzed, and in 94% of the cells the long arm of the *Ag. intermedium* chromosome was paired with the 7Ai-2L segment of the translocation chromosome either as a heteromorphic T2AS · 2AL-7Ai-2L//7Ai-2L · 7Ai-2S rod bivalent (87%) or in the form of open 2AL · 2AS//T2AS · 2AL-7Ai-2L//7Ai-2L · 7Ai-2S trivalents (7%). A critical C-banded bivalent and trivalent is shown in Fig. 6a, b. The *Ag. intermedium* segment was unpaired in only 6% of the PMC's analyzed, either as 2AL · 2AS//T2AS · 2AL-7Ai-2L rod bivalents (3%) or as univalents (3%). The chiasmata connecting the two 7Ai-2L segments of the complete 7Ai-2 chromosome and the T2AS · 2AL-7Ai-2L translocation were frequently found to be located in interstitial regions of the long chromosome arms (Fig. 6b). Furthermore, the meiotic analysis confirmed that W44 and W49 differ from each other with respect to the presence of T5BS · 6BS and T5BL · 6BL translocations. A critical quadrivalent involving chromosomes 5B, 6B, T5BS · 6BS and T5BL · 6BL is shown in Fig. 6c.

Isozyme analysis

Isozyme analysis of *a*-AMY-2 and PER-4 showed the absence of wheat bands in lines W44 and W52, but not in W49, and no novel bands associated with 7Ai-2 were found. The missing isozyme of W44 and W52 had similar isoelectric points (pIs) to *a*-AMY-D2 and PER-D4, and α-AMY-A2 and PER-A2 of 'Chinese Spring' wheat (using aneuploid analysis, results not shown) and indicated the loss of chromosomes 7D and 7A in the respective lines. A novel isozyme band was found in EP-1 gels. From Fig. 7 it can be seen that 'Heine IV', the backcross parent, has three EP-1 isozymes. The most anodal band is missing in line W44, and the most cathodal band is absent in W52. These bands correspond to the alleles *Ep-D1a* and *Ep-A1b* of chromosomes 7D and 7A, respectively (Koebner et al. 1988; R.M.D. Koebner, personal communication). In both substitution lines a novel band was found cathodal to the middle EP-B1 band; this therefore corresponds to the alien Ep-Ai1 allele. Line W49 showed the normal three-banded phenotype of 'Heine IV', and we concluded that *Ep-Ai1* is not present in this translocation line.

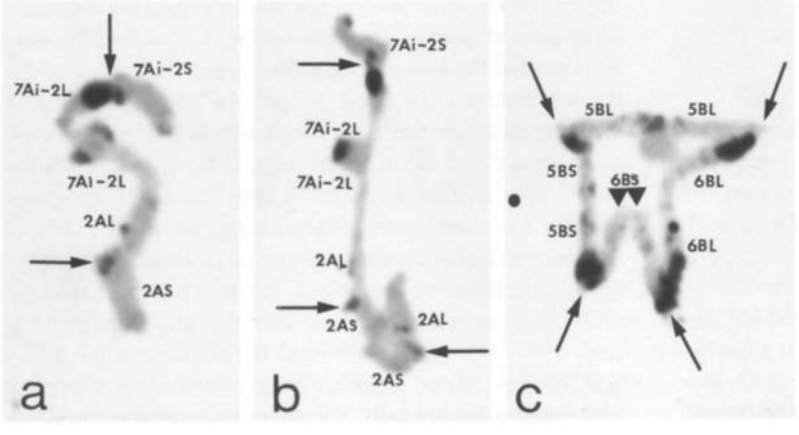

Fig. 6. C-banding patterns of the critical meiotic chromosome configurations observed in the F_1 hybrid of the cross W44 × W49: **a** T2AS · 2AL-7Ai-2L//7Ai-2L · 7Ai-2S bivalent, **b** 2AL · 2AS// T2AS · 2AL-7Ai-2L//7Ai-2L · 7Ai-2S trivalent and **c** quadrivalent involving chromosomes 5B, 6B, T5BS · 6BS and T5BL · 6BL (*arrows* point to the centromeres)

Table 1. Leaf rust, stem rust and stripe rust reactions of wheat-*Agropyron intermedium* derivatives T2, W44, W49 and the recipient wheat cv 'Heine IV'

Line	Leaf rust						Stem rust						Stripe rust
	14	14	61	53	77	104–	21	11	294	34	102	34–	108E141A+
		SaBa		SaBa	SaBa	2,3,6,7						1,2,3,6,7,8,9	
T2 addition	0;	0;	0;	;	0	0;=	;	1	1	1+	;	2–	;N
7Ai-2(7D) substitution W44	;	0;	;	;	0;	0;	;1	1–2	1+	1+	;1	2	0;
T2AS · 2AL-7Ai-2L translocation W49	;	;	;	;	;	0;	3	3	3	3	3–	3+	4
'Heine IV'	3	3–	3–	–	3+	–	–	3+	3+	–	3+	–	–

–: Not tested

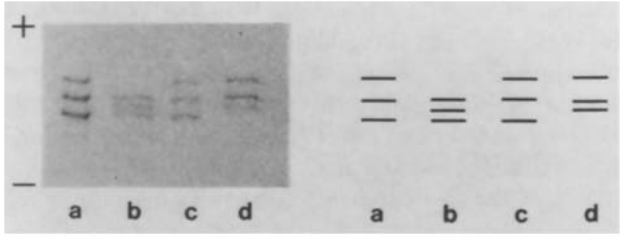

Fig. 7. Variation for EP-1 isozymes: **a** 'Heine IV', **b** W44, **c** W49, **d** W52

Resistance analysis

The parent wheat cv 'Heine IV' and lines T2, W44 and W49 were tested for their reactions to leaf rust, stripe rust and stem rust. The results show that 'Heine IV' is susceptible to all tested races of the leaf rust and stem rust pathogens, whereas the chromosome addition line T2 and the 7Ai-2(7D) substitution line W44 are highly resistant to all three rust pathogen species. However, only resistance to leaf rust is expressed in the T2AS · 2AL-7Ai-2L translocation line W49 (Table 1).

Discussion

C-banding analysis of lines W44 and W52 showed that wheat chromosome pairs 7D or 7A, respectively, are missing and replaced by a complete chromosome pair of *Ag. intermedium*. The loss of chromosomes 7A and 7D is compensated by the presence of the alien chromosome pair, which indicates that the *Ag. intermedium* chromosome belongs to group 7 of the *Triticeae*. This was also confirmed by isozyme analysis. Both substitution lines lack their respective endopeptidase isozymes, but they do show the presence of an alien *Ag. intermedium* band. Since endopeptidase genes are located on the long arms of group 7 homoeologues, the absence of wheat bands and the presence of new EP-1 bands support the likelihood of a homoeologous substitution.

Wienhues (1971, 1979) assumed that the *Ag. intermedium* chromosomes present in W44 and W52 that carry the genes for resistance to leaf rust, stripe rust and stem rust were related to homoeologous group 7 and that wheat chromosomes belonging to this group were also involved in the selected radiation-induced chromosome translocations. However, Wienhues' data based on meiotic pairing analysis of F_1 hybrids in testcrosses with marker lines were highly conflicting. The C-banding and isozyme data presented here show clearly that the *Ag. intermedium* chromosome of W44 and W52 are homoeologous to group 7.

The *Ag. intermedium* chromosomes present in lines W44 and W52 are different from an *Ag. intermedium* chromosome added to the chromosome complement of hexaploid wheat cv 'Vilmorin 27'. This material was selected by Y. Cauderon in backcross families of a partial amphiploid *T. aestivum-Ag. intermedium* with 'Vilmorin 27' (Cauderon 1966; Cauderon et al. 1973). The and Baker (1970) showed that the *Ag. intermedium* chromosome pair present in the addition line L1 (=TAF2) compensates for the loss of chromosome pair 7D and that the *Ag. intermedium* chromosome pair of line L2 (=TAF1) compensates for the loss of chromosome pair 3A of wheat in derived substitution lines, indicating homoeology of these chromosomes to group 7 and 3, respectively. The homoeologous relationships of all the added *Ag. intermedium* chromosomes was established by analyzing chromosome and plant morphology and storage protein composition, and by isozyme analysis (Forster et al. 1987). The results showed that chromosome addition line L1 carried a group 7 *Ag. intermedium* chromosome pair. Consequently, the added chromosome present

in the 'Vilmorin 27' chromosome addition line L1 and the *Ag. intermedium* chromosomes found in W44 and W52 were designated 7Ai-1 and 7Ai-2, respectively, to show they were not the same. Disease resistance analysis of the 'Vilmorin 27'-*Ag. intermedium* addition lines showed that 7Ai-1 present in line L1 conditions resistance to stem rust, whereas chromosomes 3Ai-1 (present in line L2) and 6Ai-1 (present in line L7) carry genes for resistance to leaf rust and stripe rust, respectively (Cauderon and Rhind 1976). Thus, the leaf rust resistance gene present in the 7Ai-2(7D) substitution line W44 and in the derived T2AS · 2AL-7Ai-2L translocation line W49 is different from that located on chromosome 3Ai-1 of the *Ag. intermedium*-'vilmorin 27' addition line L2.

Although 7Ai-1 and 7Ai-2 belong to the same homeologous group, 7Ai-1 is different from 7Ai-2. This is further supported by the lack of meiotic chromosome pairing at metaphase I (Zeller, unpublished results) and also by differences in C-banding pattern (Friebe et al. unpublished results). 7Ai-1 is almost completely euchromatic and shows only one very small C-band in each chromosome arm. These differences indicate that the 7Ai-1 and 7Ai-2 chromosomes originate from different genomes. Furthermore, while the additional *Ag. intermedium* EP-1 isozyme band found in W44 and W52 is missing in the 'Vilmorin 27'-*Ag. intermedium* addition line L1, L1 shows *Ag. intermedium* bands for α-AMY-2 and PER-4 (Forster et al. 1987) that are not present in lines W44 and W52, again suggesting that 7Ai-1 and 7Ai-2 were derived from different genomes. However, chromosome 7Ai-2 of lines W44 and W52 is identical both in morphology and C-banding pattern to a chromosome pair designated C present in the donor species *Ag. intermedium* (Friebe et al. unpublished results). This indicates that 7Ai-2 was not structurally rearranged compared to the corresponding chromosome of the ancestral species.

The combined C-banding and ISH analyses revealed the presence of a wheat-*Ag. intermedium* T2AS · 2AL-7Ai-2L chromosome translocation in W49 and showed that the translocation chromosome consists of the complete short arm of wheat chromosome 2A, 74% of the proximal region of the long arm of 2A and 48% of the distal region of the long arm of the *Ag. intermedium* chromosome 7Ai-2. The presence of a distally located large segment derived from the *Ag. intermedium* chromosome arm 7Ai-2L in the translocation chromosome was demonstrated by meiotic pairing analysis. The chiasmata connecting the two 7Ai-2L segments of the complete 7Ai-2 chromosome and the T2AS · 2AL-7Ai-2L translocation were frequently observed in interstitial regions of the long chromosome arms. Because crossing over and chiasma formation under normal conditions in wheat is restricted to homologous chromosomes, the meiotic data show that not only the telomeres but the entire distal region of the long arm of the T2AS · 2AL-7Ai-2L translocation is derived from the *Ag. intermedium* chromosome 7Ai-2.

The chromosomes involved in this translocation belong to different homoeologous groups and therefore, the transferred 7Ai-2L segment cannot compensate for the loss of the missing 2AL segment. Because the missing 2Al segment has a size of only 1.4 μm, this partial loss of wheat chromatin may be tolerated and the agronomic performance of line W49 may not be impaired. The breakpoint of the T2AS · 2AL-7Ai-2L translocation is located in an interstitial region of the long arm indicating that this translocation was not produced by the common centric breakage-fusion mechanism but may have been induced by the mutagenic X-ray treatment used in its production.

It was suggested by Wienhues (1973) that the genes for resistance to leaf rust, stripe rust and stem rust were not located on the same arm of the *Ag. intermedium* chromosome. It was assumed that the genes for leaf rust and stem rust resistance were located on one arm, with the former at a proximal and the latter at a distal position, whereas the stripe rust resistance gene was located on the opposite arm. We found no evidence to support this assumption. The data presented here show that the 7Ai-2(7D) substitution line W44 is resistant to leaf rust, stripe rust and stem rust and that only the leaf rust resistance is expressed in the T2AS · 2AL-7Ai-2L translocation line W49. This suggests that the gene(s) conferring resistance to leaf rust is located in the translocated *Ag. intermedium* segment of 7Ai-2L, whereas the genes for stem rust and stripe rust resistances are located either in the short arm of chromosome 7Ai-2 or in the proximal region of the long arm.

Line W49 carries excellent leaf rust resistance dervied from *Ag. intermedium* in the form of a cytologically stable wheat-*Ag. intermedium* chromosome translocation. Because crossing-over is restricted to homologous chromosome regions in wheat, segregation of leaf rust resistance in crosses with other cultivars should be normal. We propose the gene symbol Lr38 for this leaf rust resistance gene derived from a group 7 *Ag. intermedium* chromosome. This type of analysis is crucial to directed chromosome engineering of wheat lines aimed at producing superior rust resistance cultivars.

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