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Pilar Rubio, Lourdes Daza, Nicolás Jouve. Meiotic behaviour, chromosome stability and genetic analysis of the preferential transmission of 1B-1R, 1A-1R and 1R(1D) chromosomes in intervarietal hybrids of wheat. *Agronomie*, 1999, 19 (1), pp.57-68. <hal-00885913>

HAL Id: hal-00885913

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Submitted on 11 May 2020

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Meiotic behaviour, chromosome stability and genetic analysis of the preferential transmission of 1B-1R, 1A-1R and 1R(1D) chromosomes in intervarietal hybrids of wheat

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(Received 16 July 1998, accepted 16 November 1998)

Abstract – The meiotic behaviour and relative transmission of segregant chromosomes was studied in the progeny of the F_1 hybrids formed between a normal common wheat and lines which contained single or double wheat-rye translocations 1AL-1RS, 1BL-1RS and/or the substitution 1R(1D). Chromosome stability was evaluated in the F_2 progenies by the analysis of different markers: C-bands, LMW- and HMW-glutenins, gliadins and secalins coded for by genes *GluA1*, *GluB1*, *GluD1*, *GluR1*, *GluA3*, *GluB3*, *GliA1*, *GliB1* and *GliR1*, located on either the short or long arm of the chromosomes of homoeologous group 1. Direct analysis of chromosome constitution and the χ^2 test, used in the genetic analysis of genes located in each chromosome arm, indicated that observed frequencies were significantly different from the expected Mendelian values in hybrids with only one translocation. The segregation patterns were biased in favour of the transmission of normal 1A over 1AL-1RS, normal 1B over 1BL-1RS, arm 1DL over 1RL and arm 1RS over 1DS. However, the same genes segregated in a Mendelian fashion in other hybrids with normal constitutions for these chromosomes or which showed double structural heterozygous constitution. The different response to the selection of different chromosomes or chromosome arms may be the product of an integrated contribution of meiotic behaviour plus differences in the vigour of male gametes carrying a normal chromosome compared to those carrying rye chromatin. However, it might also be due to differences in viability once male and female gametes combine. (© Inra/Elsevier, Paris.)

Triticum aestivum / wheat/rye translocations / HMW-glutenins / LMW-glutenins / secalins / gliadins

Résumé – Comportement méiotique, stabilité chromosomique et analyse génétique de la transmission préférentielle des chromosomes 1B-1R, 1A-1R et 1R(1D) dans des hybrides intervariétaux de blé tendre. Le comportement méiotique et la transmission des chromosomes en ségrégation ont été étudiés dans la descendance d'hybrides F_1 entre un blé tendre de constitution normale et des lignées qui portaient des translocations 1AL-1RS, 1BL-1RS et/ou la substitu-

Communicated by Hervé Thiellement (Genève, Switzerland)

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tion 1R(1D). La stabilité chromosomique a été évaluée dans les descendance F₂ par l'analyse de différents marqueurs : les bandes C, les gluténines de hauts et de faibles poids moléculaires (HMW et LMW), les gliadines et les sécaldines codées par les gènes *GluA1*, *GluB1*, *GluD1*, *GluR1*, *GluB3*, *GliA1*, *GliB1* et *GliR1*, localisés soit sur le bras court soit sur le bras long des chromosomes homéologues du groupe 1. L'analyse directe de la constitution chromosomique et le test du χ^2 , pour l'analyse génétique des gènes localisés sur chacun des bras chromosomiques, a montré que les fréquences observées diffèrent de celles attendues pour une ségrégation mendélienne, chez les hybrides porteurs d'une seule translocation. Les ségrégations étaient biaisées en faveur de la transmission du 1A normal versus 1A-1RS, de 1B normal versus 1B-1RS, du bras 1DL versus 1RL et du bras 1RS versus 1DS. Cependant les mêmes gènes ségrègent de façon mendélienne chez les hybrides de constitution normale ou porteurs de la double translocation. La réponse différente à la sélection de ces chromosomes ou bras chromosomiques pourrait résulter de la combinaison du comportement méiotique et de la différence de vigueur des gamètes mâles normaux vis-à-vis de ceux porteurs de chromatine de seigle. Cependant cela pourrait également refléter des différences de viabilité après la fécondation. (© Inra/Elsevier, Paris.)

Triticum aestivum / translocations blé-seigle / gluténines HPM/ gluténines FPM/ sécaldines/ gliadines

1. INTRODUCTION

Wheat cultivars carrying rye chromatin have been available for decades. Spontaneous chromosome 1B/1R substitution lines were first found in old German wheat varieties [25, 35, 36]. Thereinafter, the substitution of chromosome 1B by the homoeologous 1R of rye was shown to be important in improving yield [19, 28], androgenetic response [12, 13, 29], resistance to different diseases [6, 8, 30] and insect attack [9, 14, 23, 33]. Consequently, there has been much interest in introducing rye genetic variation into wheat, and different methods have been used to make partial or total transfers of chromosome 1R. The most useful method is direct introduction using recurrent backcrosses after wheat-rye interspecific hybridisation. Indirect introgression is an alternative, using the synthetic amphiploid triticale or substitution or addition lines of wheat carrying the alien chromosome as bridging species. All these methods require a number of backcrosses to reconstitute the genotype of the recipient parent. Such are the origins of the winter wheat cultivars 'Amigo' and 'Kavkaz' which have chromosome 1A substituted by 1AL-1RS and 1B by 1BL-1RS, respectively [3, 25, 37]. These lines have been used to introduce the short arm of 1R into other wheats [36].

The ease of obtaining rye-wheat translocated or substituted lines from F₁ hybrids between a normal recipient and a donor carrying the alien chromatin

depends on chromosome behaviour in the early hybrid generations. Due to the *Ph* gene effect [32], chromosome pairing is usually restricted to homologous chromosome segments in F₁ hybrids of wheat heterozygous for rye alien introgression. Wheat-rye pairing frequency is typically low in the presence of this gene [7, 16, 20, 27]. However, induction of pairing and recombination between rye chromosome 1R rye and its wheat homoeolog has been achieved by Koebner and Shepherd [17] using wheats with the *ph1b* mutant allele or which lacked chromosome 5B.

Marker genes can be used to accelerate the reconstitution of the genotype of the recurrent parent in plants that maintain alien chromatin during backcrossing. This methodology was utilised to obtain rye/wheat substitutions 1R(1A), 1R(1B) and 1R(1D) and translocations 1AL-1RS, 1BL-1RS [32]. Koebner and Shepherd [17, 18] used storage protein genes and prominent telomeric C-bands as markers to follow rye chromosome arms 1RS and 1RL in wheat recombination lines. These authors observed distorted segregation in favour of the transmission of gametes carrying normal wheat chromosomes and also the competitive disadvantage at fertilisation of gametes carrying 1R/1D translocated chromosomes. It would be of interest to know the factors that influence the transmission of the rye chromatin to the progeny.

In this study an investigation was made into both meiotic pairing and chromosome transmission in

single or double heterozygous combinations of 1AL-1RS, 1BL-1RS and 1R chromosomes from the F_1 to the F_2 of several wheat hybrids. The plant material studied is currently involved in a project designed to introduce the short arm of 1RS, or the whole chromosome 1R, into Spanish wheat, using several wheat donors with different combinations of the substitutions and/or translocations. Chromosome stability was analysed using easily scorable chromosomal and genetic markers, namely C-bands, LMW- and HMW-glutenins, gliadins and secalins.

2. MATERIALS AND METHODS

Crosses were made between the Spanish wheat cultivar 'Pané-247' (female parent) and six rye-containing wheat varieties (male parent). Lines involving translocations or substitutions 1AL-1RS(1A), 1BL-1RS(1B) and 1R(1D) were kindly provided by Dr J.P. Gustafson. They were: 1) 'Anza/S149' (hereafter referred to as S149), a 1R(1D) substitution line derived from the common wheat 'Anza' and the triticale '6A250' [10]; 2) '48-141', a selected line derived from the cross between 'Amigo' ($2n = 20'' + 1AL-1RS''$) and 'Glennson 81' ($2n = 20'' + 1BL-1RS''$), which contains a 1AL-1RS translocation; 3) '88-31', a selected line derived from the cross between 'S149' and 'Amigo', which contains a 1AL-1RS translocation; 4) '48-136', a selected line derived from the cross between 'Amigo' and 'Glennson 81', which contains a 1BL-1RS translocation; 5) '48-145', a selected line derived from the cross between 'Amigo' and 'Glennson 81', which contains both translocations 1AL-1RS and 1BL-1RS, and 6) '7841-34', a selected line derived from the cross between 'S149' and 'Glennson 81', which contains a 1BL-1RS translocation and a 1R(1D) substitution. These lines were formerly used in experiments examining rye gene expression in a wheat background [4, 5, 11]. All these lines were utilised as parentals to generate segregating material and compare the efficiency in producing similar arm or chromosome substitutions in the Spanish selected cultivar 'Pané-247'. The F_2 families from the hybrids 'Pané-247' \times '48-141', 'Pané-247' \times '88-31', 'Pané-247' \times '48-136', 'Pané-247' \times 'S-149', 'Pané-247' \times '48-145-1' and 'Pané-247' \times '7841' (hereafter referred to as fam-1 to fam-6, respectively) were investigated for the segregation of prolamin markers and in some of them for the chromosome constitution.

C-banding [16] was used to identify the rye-wheat chromosome configurations during the meiosis of pollen mother cells (PMC) in three F_1 hybrids: 'Pané-247' \times 'S149' (hemizygous for 1R and 1D), 'Pané-247' \times '48-145' (double heterozygous for 1A-1AL-1RS and 1B-1BL-1RS), and 'Pané-247' \times '7841-34' (hemizygous for 1R and 1D and heterozygous for 1B-1BL-1RS). To elucidate the chromosome constitution somatic metaphase cells were C-banded using the method of Lukaszewski and Gustafson [22] in samples of fam-1, fam-2 and fam-3.

Progeny obtained by selfing each of the above hybrids were screened for their glutenin, gliadin and secalin constitution using the genes *GluA1*, *GluB1*, *GluD1*, *GluR1*, *GluA3*, *GluB3*, *GliB1*, *GliD1* and *GliR1* as markers. In order to perform the analysis of endosperm proteins without interfering with germination, a piece of the endosperm containing no part of the embryo was separated out. This was ground and the proteins extracted as described by Singh and Shepherd [32]. Prolamins were analysed using vertical polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) at different concentrations: 10 % for gliadins and secalins, and 12 % for glutenins. Similar samples corresponding to several standard wheats were added as controls ('Chinese Spring', 'Bezostaya', 'Hope' 'Champlein', 'Ablaca'). A molecular weight standard (Sigma 6H) was used which contained myosin (205.000), β -galactosidase (116.000), phosphorylase B (97.000), bovine albumin (66.000), egg albumen (45.000) and carbonic anhydrase (29.000). Electrophoresis was performed at 16 mA/gel for 16–17 h at room temperature. Gels were stained with 1 % Coomassie Brilliant Blue R-250 in ethanol. Parentals, controls and segregant samples of the same progeny were included on the same gel. In order to obtain more gliadin and secalin markers, a second analysis was performed using 7 % acid polyacrylamide gel electrophoresis (A-PAGE) in aluminium lactate buffer following the procedure of Lafiandra and Kasarda [30] with minor modifications.

3. RESULTS

3.1. F_1 meiotic behaviour

The meiotic behaviour at metaphase I in three hybrids was investigated (*table II*) presented as chromosome configurations and mean number of chiasmata per bivalent. The meiotic behaviour of

Table I. Pairing configurations at metaphase I of PMCs analysed by C-banding in three hybrids intervarietal hybrids of common wheat heterozygous for rye-wheat translocations or substitutions.

Hybrids F ₁ :	Pané × 48-145	Pané × 7841	Pané × S-149
Chromosome Constitution	19'' + 1A' + 1AL-1RS' + + 1B' + 1BL-1RS'	19'' + 1B' + 1BL-1RS' + + 1R' + 1D'	20'' + 1R' + 1D'
N° PMC	93	30	35
21 bivalents	49	0	0
19 biv. + 1 Quadriv. (1B/1BL-1RS/1RS-1AL/1A)	4	0	0
19 biv + 1 triv + 1 univ (1BL-1RS/1AL-1RS/1A) + 1B	5		
(1B/1BL-1RS/1R) + 1D		4	
20 biv + 2 univ (1A'+1AL-1RS')	2		
(1R' + 1D')		22	29
(1D' + 1B')		2	
20 biv + 2 univ (1B'+1BL-1RS')	8	0	
20 biv + 2 univ (1R as biv))	12		
19 biv. + 4 univ	9	2	6
Xata/Biv.	1.75 ± 0.3	1.55 ± 0.4	1.45 ± 0.3

chromosomes involved in translocations or substitutions in the F₁ could explain the types of chromosome constitutions and gene combinations expected in the F₂. It is of interest to note that the hybrids studied showed a meiotic pairing almost that expected for each chromosome constitution, assuming a strictly homologous pairing (*figure 1a*).

The heterozygous chromosomes: 1AL-1RS/1A, 1BL-1RS/1B and 1R/1D, show the following meiotic behaviour:

– as open bivalents paired by the homologous arm 1AL or 1BL in hybrids with only one translocation (fam-1, fam-2, fam-3). Open bivalents associated with 1RS were also found at a lower frequency in hybrids carrying both translocations ('Pané-247' × '48-145') or the translocation 1BL-1RS and the substitution 1R(1D) (fam-6);

– as univalents, owing to the lack of pairing or precocious separation of homologous paired regions. Chromosomes 1R and 1D, present in single doses in fam-4, were always observed as univalents;

– as multivalents, owing to pairing by the homologous arm 1RS of chromosomes belonging to different pairs. This arm is easily identified by C-banding and acts as a nexus for alternate pairing and co-orientation of the two translocation chromosomes (quadrivalents 1AS-1AL/1AL-1RS/1RS-1BL/1BL-1BS), or a translocation and the whole 1R chromosome (trivalents 1BS-1BL/1BL-1RS/1RS-1RL). Moreover, the frequency of multivalents was similar to that of open bivalents joining both translocated chromosomes by 1RS (1AL-1RS/1RS-1BL). The low frequency of these figures can be explained by desynapsis during prophase.

Other results of interest were the following: 1) the mean number of chiasmata per bivalent was greater than 1 in all cases; 2) a predominance of ring bivalents with two or more chiasmata between homologous pairs not involved in translocations or substitutions, were observed.

3.2. F₂ progeny: chromosome constitution

The genetic analysis of the progenies was preceded by a screening of a series of polymorphic

Table II. Segregation of the critical chromosomes in the F₂ of three hybrids of common wheat heterozygous for rye-wheat translocations or substitutions (1BLr and 1Br means recombinant chromosomes by interchange between centromere and the C-band L2.3 located in the long arm 1BL).

Hybrid F ₁	F ₂		
	2n	Chromosome constitution	% total cells
'Pané x 48-136' 2n = 42 20'' + 1B' + 1BL-1RS'		Fam-3	
	42	20'' + 1B''	28.1
	42	20'' + 1BL-1RS''	6.8
	42	20'' + 1B' + 1BL-1RS'	31.8
	42	20'' + 1BLr-1RS' + 1BL-1RS'	5.3
	42	20'' + 1B' + 1BLr-1RS'	5.3
	42	20'' + 1B' + 1Br'	5.3
	42	20'' + 1B' + telo 1RS	3
	42	20'' + 1Br' + 1BLr-1RS'	0.8
	42	20'' + 1Br' + 1BL-1RS'	3.7
	42	20'' + 1Br''	1.5
	42	20'' + 1BLr-1RS' + iso1BL	0.8
	43	20'' + 1B' + 1Br' + 1BLr-1RS'	1.5
	43	20'' + 1B' + 1BL-1RS' + telo 1RS	0.8
	41	20'' + 1B'	4.5
	41	20'' + 1BLr-1RS'	0.8
'Pané x 48-145' 2n=42 19'' + 1A' + 1AL-1RS' + + 1B' + 1BL-1RS'		Fam-5	
	42	19'' + 1A' + 1AL-1RS' + 1B' + 1BL-1RS'	28.1
	42	19'' + 1A'' + 1BL-1RS''	9.4
	42	19'' + 1AL-1RS'' + 1BL-1RS''	6.25
	42	19'' + 1AL-1RS'' + 1B''	6.25
	42	19'' + 1AL-1RS'' + 1Br''	6.25
	42	19'' + 1AL-1RS'' + 1B' + 1BL-1RS'	6.25
	42	19'' + 1A' + 1AL-1RS' + 1Br''	6.25
	42	19'' + 1A' + 1AL-1RS' + 1B' + 1Br'	3.1
	42	19'' + 1A' + 1AL-1RS' + 1Br' + 1BL-1RS'	3.1
	42	19'' + 1A' + 1AL-1RS' + 1B' + 1BLr-1RS'	3.1
	42	19'' + 1A'' + 1B' + 1BL-1RS'	3.1
	42	19'' + 1A' + 1AL-1RS' + 1B' + 1BLr-1RS'	3.1
	42	19'' + 1AL-1RS'' + 1B' + 1Br'	3.1
	42	19'' + 1A'' + 1BL-1RS' + 1BLr-1RS'	3.1
	42	19'' + 1A' + 1AL-1RS' + 1B' + iso1BLr'	3.1
41	19'' + 1AL-1RS' + 1B' + 1Br'	3.1	
'Pané x 7841' 2n = 42 19'' + 1B' + 1BL-1RS' + + 1D' + 1R'		Fam-6	
	42	19'' + 1B'' + 1R''	24.3
	42	19'' + 1B' + 1BL-1RS' + 1D' + 1R'	16.2
	42	19'' + 1BL-1RS'' + 1D''	8.1
	42	19'' + 1BL-1RS'' + 1R''	2.7
	42	19'' + 1BL-1RS'' + 1D''	2.7
	42	19'' + 1B' + 1BL-1RS' + 1R''	2.7
	42	19'' + 1Br' + 1BLr-1RS' + 1D' + 1R'	2.7
	44	19'' + 1B'' + 1D'' + 1R''	8.1
	43	19'' + 1B'' + 1D' + 1R''	8.1
	43	19'' + 1B' + 1BL-1RS' + 1D'' + 1R'	2.7
	43	19'' + 1B' + 1Br' + 1D' + 1R''	2.7
	43	19'' + 1B' + 1BL-1RS' + 1D' + 1R' + telo1RL	2.7
	41	19'' + 1B' + 1D' + 1R'	2.7
	41	19'' + 1B' + 1BL-1RS' + 1R'	2.7
	41	19'' + 1BL-1RS'' + 1D'	2.7
41	19'' + 1BLr-1RS'' + 1D'	2.7	
41	19'' + 1B' + 1BL-1RS' + 1DS-1RL'	2.7	
41	19'' + 1B' + 1R' + telo1RL	2.7	

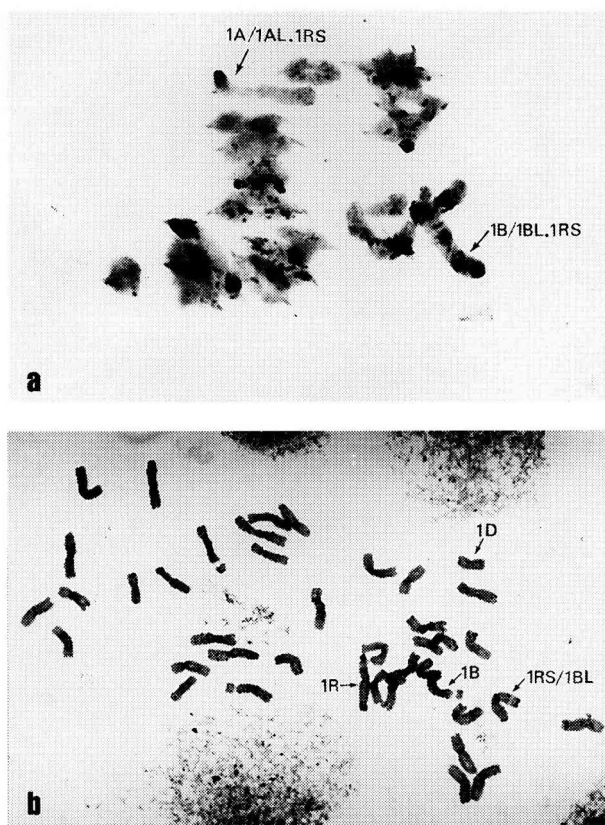


Figure 1. a) Chromosome pairing and identification by C-banding of chromosomes involved in translocations in PMCs of the hybrid 'Pané-247' × '48-145-8' ($2n = 42; 19'' + 1A' + 1AL-1RS' + 1B' + 1BL-1RS'$). The open bivalents 1A/1AL-1RS and 1B/1BL-1RS are shown. b) Microphotograph of a somatic metaphase plate of a plant belonging to the F_2 progeny after the cross 'Pané-247' × '7841-34-4'

systems, including prolamin genes and C-bands. With respect to the latter, the chromosomes 1B, 1D, 1RS-1AL, 1RS-1BL and 1R of the parental lines showed distinctive C-banding patterns. Chromosome 1A was unique in showing no band in the parental lines. The whole chromosome 1R and/or the translocated arm 1RS showed patent C-bands that were easily distinguished in the progenies (figure 1b). Chromosome 1R showed a duplication in the telomeric band on arm 1RL.

An important polymorphic C-band was found in the long arm of chromosome 1B. 'Pané-247' shows a distinctive interstitial C-band in the region L2.3

that was absent from both the translocated chromosome 1BL-1RS and the whole 1B of the rye donor parentals used in the crosses. The heterozygous constitution of 1BL2.3 was very evident in the F_1 plant hybrids. The scoring of the three structural constitutions of this arm, or any other spontaneous aberrations, presented no difficulties in the F_2 plants. Moreover, recombinant chromosomes in the region between the centromere and band L2.3 were detected in the F_2 of the hybrids analysed. The F_1 is double heterozygous for the short arm and band L2.3: $1BS-1BL^{2.3+} / 1RS-1BL^{2.3-}$ in the hybrids studied. Both parental chromosome types were the most frequent in the progeny. However, chromosome types $1BS-1BL^{2.3-}$ and $1RS-1BL^{2.3+}$ were found in the progeny of the F_1 as a consequence of recombination between the centromere and locus L2.3 during meiosis (1BLr in table II).

The data on the chromosome constitution of three F_2 derived families are recorded in table II. In all cases, rather high stabilities and balanced transmissions were detected for chromosomes not involved in translocations or substitutions. However, the critical chromosomes segregated in a distorted fashion as demonstrated not only by the direct analysis of chromosome constitution but also by the segregation of marker genes as shown below. Segregation was biased in favour of 1B over 1BL-1RS in at least two F_2 families: 64.3 % 1B and 35.7 % 1BL-1RS in fam-3, and 63.9 % 1B and 36.1 % 1BL-1RS in fam-6. However, a different situation was found for the relative transmission of 1A (41.3 %) and 1AL-1RS (58.7 %), and 1D (39.7 %) and 1R (60.3 %), in fam-5 and fam-6, respectively. Telocentrics, isochromosomes and fusion-centrics of non-parental arm combinations were also observed. Their frequency was higher in the fam-5 and fam-6 progenies which have two critical pairs.

3.3. F_2 progeny: segregation of prolamin genes

Electrophoretic analyses of polymorphic systems on parental lines were performed using wheat varieties previously investigated as controls to identify

the alleles of a series of gene markers located on each arm of the chromosomes of homoeologous group 1. The genes were selected under criteria of existence of allelic differences between the parentals and easy distinction of heterozygotes (table III and figure. 2). They include the HMW-glutenins coded for by genes *Glu-A1*, *Glu-B1*, *Glu-D1* and *Glu-R1*, located, respectively, on the homoeologous arms 1AL, 1BL, 1DL and 1RL, the LMW-glutenins of the *Glu-A3* and *Glu-B3* genes located on the short arms 1AS and 1BS, respectively, and the gliadins encoded by *Gli-B1* and *Gli-D1*, located, respectively, on the short arms 1BS and 1DS. Segregation of all these genes in the F₂ offspring of each hybrid was further studied (table IV).

The genes *GluA1* (HMW-glutenin), *GluB3* (LMW-glutenin) and *GliD1* (w-gliadin), differentiate 'Pané-247' and some rye donor lines from the others due to the existence of null allelism (*GluA1*, studied by SDS-PAGE) or by the masking of some of the products by other proteins in the gels (*GluB3* and *GliD1*, investigated by SDS-PAGE and A-PAGE, respectively). In these cases there was dominance and the scoring of only two phenotypic classes was possible. The same applies for the genes located in 1AS, 1BS and/or 1DS which are found in single doses in the F₁ hybrids with two doses of 1RS (fam-5 and fam-6).

The progenies scored for each gene were tested for Mendelian segregation. The χ^2 analysis of the data indicated that the observed frequencies were not significantly different from the 1:2:1 (codominant) or 3:1 (dominant) ratios expected for coding genes located in chromosomes not involved in translocations or substitutions (last column in table IV). However, the segregation of genes located in critical chromosomes was distorted and deviated significantly from the Mendelian. Such was recorded for the following genes or chromosome markers: *GluA3* over *GliR1* in fam-1 and fam-2 (F₁: 2n = 20'' + 1AL-1AS' + 1AL-1RS'); *GliB1* and *GluB3* over *GliR1* in fam-3 (F₁: 2n = 20'' + 1BL-1BS' + 1BL-1RS'); *GliD1* over *GliR1* and *GluD1* over *GluR1*, in fam-4 (F₁: 2n = 20'' + 1D' + 1R'); and *GluB1*, in fam-6 (F₁: 2n = 19'' + 1BL-1BS' + 1BL-1RS' + 1D' + 1R'). If the translocated chromosome 1AL-1RS or 1BL-1RS and the normal 1A or 1B segregated independently in hybrids carrying a single translocation, a 1:2:1 ratio of homozygous normal: translocation heterozygote: translocation homozygote would be expected in the F₂, for each. The χ^2 test indicated that the observed frequencies were significantly different from the Mendelian ratio for the genes located in the critical chromosomes in the mentioned hybrids. However, the same genes segregated in a Mendelian fashion in other hybrids with normal constitutions for these chromosomes. The involvement of the chromosome constitution in the

Table III. Catalogue of genes and alleles present in the parental lines used in the hybrids

Chromosome arms	Genes	'Pané 247' 21''	'48-141' 20'' + 1AL-1RS	'88-31' 20'' + 1AL-1RS	'48-136' 20'' + 1BL-1RS	'S-149' 20'' + 1R(1D)	'48-145' 19'' + 1AL-1RS + 1BL-1RS	'7841-34' 19'' + 1BL-1RS + 1R(1D)
1AS	<i>GluA3</i>	LMW2			LMW2	LMW2		
1BS	<i>GliB1</i> <i>GluB3</i>	γ 1 LMW1	γ 2 null	γ 2 null		γ 2 null		
1DS	<i>GliD1</i>	SDS10 =ω3	SDS8 null	SDS8 null	SDS8 null		SDS8 null	
1RS	<i>GliR1</i>		SDS15-17	SDS15-17	SDS15-17	SDS15-17	SDS15-17	SDS15-17
1AL	<i>GluA1</i>	null	HMW2*	HMW2*	HMW2*	null	HMW2*	null
1BL	<i>GluB1</i>	HMW7 + 8	HMW7 + 9	HMW6 + 8	HMW7 + 9	HMW6 + 8	HMW7 + 9	HMW7 + 9
1DL	<i>GluD1</i>	HMW2 + 12	HMW5 + 10	HMW5 + 10	HMW5 + 10		HMW5 + 10	
1RL	<i>GluR1</i>					HMW7*		HMW7*

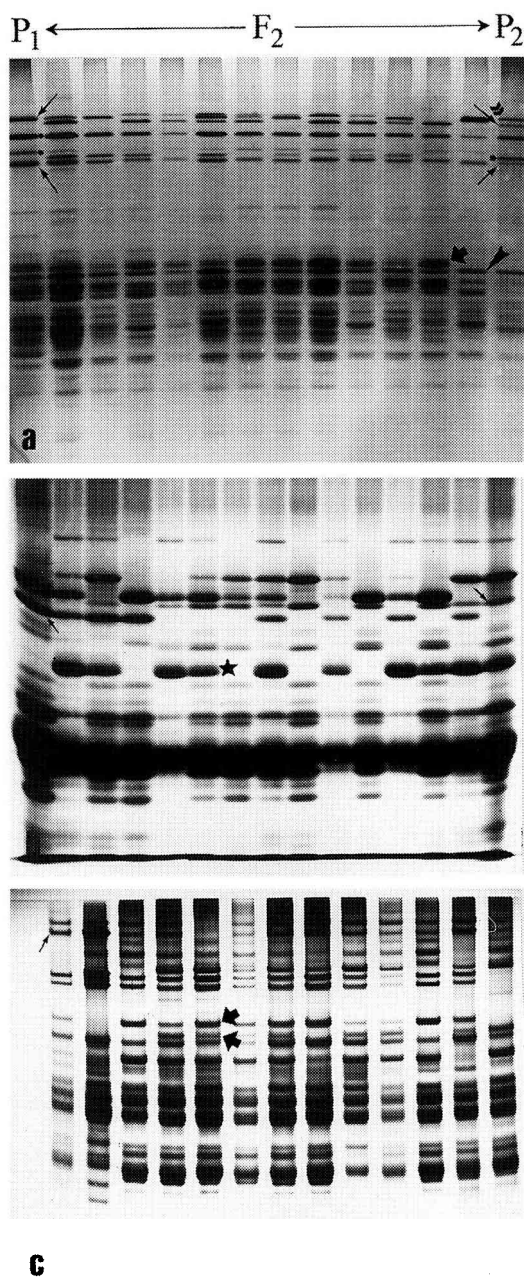


Figure 2. Electrophoretic patterns of different systems to analyse prolamines of several wheat parentals (P_1 and P_2) and plants belonging to their F_2 progeny. a) The HMW- and LMW of fam-3 ($P_1 \times P_2$) studied by 12 % SDS-PAGE; b) gliadines of Pané-247' \times '88-31' ($P_1 \times P_2$) studied by 10 % SDS-PAGE; c) gliadines of 'Pané-247' \times 'Anza/S149' ($P_1 \times P_2$) studied by 7 % acid-PAGE. Long thin arrow = HMW2+12/5+10; asterisks = HMW7+8/7+9; double tip arrow = HMW 2*; arrow head = LMW2; Black bold arrow = LMW1; stars = secalines SDS15-17; Short thin arrow = SDS8 and SDS10.

segregation of wheat or rye genes was also evident in the hybrids carrying two translocations (1AL-1RS and 1BL-1RS) or one translocation and one substitution [1BL-1RS and 1R(1D)]. The segregation of *Glu-A3* and *Gli-B1* in fam-5, and *Glu-B3* and *Gli-D1* in fam-6 were discarded from the Mendelian analysis owing to the masking segregation of the same rye marker located in two different pairs, and because of the formation of multivalents which leads to dependence in their co-segregation.

4. DISCUSSION

The hybrids used to generate the segregant progenies bear heterozygous constitution for one or two translocations (1AL-1RS/1AL-1AS or/and 1BL-1RS-1BL-1BS) or a whole chromosome (1R/1D), or a translocation and a substitution (1BL-1RS-1BL-1BS and 1R/1D). If the hybrid is heterozygous for only one translocation, the normal pairing of the homologous arms would bring the non-homologous arms to physically associate at prophase I of meiosis and thereby encourage co-orientation and segregation at the end of the first meiotic division. The hybrids studied at meiosis had the normal gene *Ph1*, which promotes homologous pairing, and lacked wheat-rye homoeologous pairing. This result is in agreement with the lack of pairing described between homoeologous chromosomes of rye and wheat due to *Ph1* [16, 20, 26, 27]. The analysis of meiosis by C-banding demonstrates that pairing is rather stable and that many cells show the maximum chromosome association possible, according to their chromosome constitution.

The high frequency of open bivalents 1A/1AL-1RS and 1B/1BL-1RS explains the emergence of recombinants between the centromere and the genes *Glu-A1*, and *Glu-B1* or the C-band 1BL2-3 located in the interstitial sites of the long arm of chromosomes 1A and 1B, respectively. Figures of 28.5 and 30.7 cM were estimated for the distance between the centromere and the loci of *Glu-A1* and *Glu-B1*, on the long arm of chromosomes 1A and 1B, respectively.

Table IV. Individual segregation of glutenin, gliadin and secalin phenotypes in six F₂ progenies of common wheat heterozygous for rye-wheat translocations or substitutions.

Hybrid	Arm	Gene a-b	aa	ab	bb	No. Plants	χ^2	P
'Pané' × '48-141' fam-1	1AS'-1RS'	<i>GluA3-GliR1</i>	17	54	9	80	11.4	< 0.01*
	1BS''	<i>GliB1</i>	18	37	27	82	2.76	> 0.3
		<i>GluB3</i>		54	28	82	3.66	> 0.1
	1DS''	<i>GliD1</i>	4	56	22	82	0.14	> 0.9
	1AL''	<i>GluA1</i>	24		58	82	0.79	> 0.3
	1BL''	<i>GluB1</i>	23	36	23	82	1.22	> 0.9
	1DL''	<i>GluD1</i>	18	43	21	82	0.42	> 0.9
'Pané' × '88-31' fam-2	1AS'-1RS'	<i>GluA3-GliR1</i>	21	34	7	61	6.9	< 0.05*
	1BS''	<i>GluB3</i>		47	12	59	0.68	> 0.3
	1DS''	<i>GliD1</i>	13	32	17	62	0.58	> 0.7
	1AL''	<i>GluA1</i>	21		40	61	2.89	> 0.1
	1BL''	<i>GluB1</i>	22	26	13	61	3.98	> 0.2
	1DL''	<i>GluD1</i>	20	21	20	61	5.92	> 0.05
	'Pané' × 48-136' fam-3	1BS'-1RS'	<i>GliB1-GliR1</i>	82	87	22	191	39.2
		<i>GluB3-GliR1</i>	82	87	22	191	39.2	< 0.001*
1DS''		<i>GliD1</i>		155	45	200	0.67	> 0.5
1AL''		<i>GluA1</i>	64		136	200	5.22	< 0.05*
1BL''		<i>GluB1</i>	71	96	33	200	14.8	< 0.001*
1DL''		<i>GluD1</i>	57	88	55	200	2.92	> 0.1
'Pané' × 'S149' fam-4	1BS''	<i>GliB1</i>	31	42	21	94	3.19	> 0.2
	1DS'-1RS'	<i>GliD1-GliR1</i>	13	69	18	100	14.9	< 0.001*
	1BL''	<i>GluB1</i>	8	73	19	100	23.6	< 0.001*
	1DL'-1RL'	<i>GluD1-GluR1</i>	32	53	15	100	6.14	< 0.05*
'Pané' × '48-145' fam-5	1DS''	<i>GliD1</i>	25	54	22	101	0.66	> 0.7
	1AL''	<i>GluA1</i>	29		72	101	0.7	> 0.3
	1BL''	<i>GluB1</i>	32	43	26	101	2.94	> 0.1
	1DL''	<i>GluD1</i>	29	50	22	101	0.7	> 0.5
'Pané' × '7841' fam-6	1AS''	<i>GluA3</i>		75	25	100	0	> 0.95
	1BL''	<i>GluB1</i>	43	40	17	100	17.5	< 0.01*
	1DL'-1RL'	<i>GluD1-GluR1</i>	21	46	29	96	1.5	> 0.2

The results show that the phenotypic fractions are biased away from expected Mendelian frequencies. A typical source of deviation is the upset in the formation or function of gametes or zygotes due to the selection of one or more genes on the chromosomes [1, 2, 14, 21]. Another possible source is the 'differential transmission' of either normal or alien chromosomes from a translocation or substitution heterozygote due to gametic or zygotic selection.

For gametic selection it has always been assumed that preferentially male gametes are affected [21, 31]. Thus, Koebner and Shepherd [17, 18] demonstrated the higher male transmission of gametes bearing 1D over those bearing 1RS-1DL or 1DS-1RL translocations in translocation heterozygotes of wheat.

The data obtained from the six F₂ reveal a different transmission of normal and alien chromosomes

depending on the type and number of heterozygous pairs. Thus, by studying the chromosomes of the progenies analysed by C-banding, or that inferred from electrophoretic markers, segregation patterns were found to be biased in favour of the transmission of:

- normal 1A over 1AL-1RS in fam-1 and fam-2;
- normal 1B over 1BL-1RS in fam-3;
- arm 1DL over 1RL and 1RS over 1DS in fam-4;
- normal 1B homozygote over 1BL-1RS in fam-6.

The first three hybrids mentioned share a single heterozygous pair (1A/1AL-1RS or 1B/1BL-1RS) that shows stable pairing through the long arm and co-orientation and separation of the short arms (1AS from 1RS, or 1BS from 1RS) which is not conditioned by other pairs. The variation observed in the transmission ratio of the long and short arms of 1D and 1R in fam-4 that was detected in the same population, could be explained in part by the production of telocentrics and other chromosome aberrations as well as their differential viability and/or capacity to fertilise. The presence of unbound chromosomes in the meiosis of this hybrid could explain the discrepancy in the transmission rates of the chromosome arms 1DS and 1DL versus 1R. Both chromosomes 1D and 1R appeared as a standard as univalents in the meiosis of the hybrid 'Pané' × 'S-149'. Laggards, mainly formed by these univalents, could misdivide at the centromere during anaphase I and produce telocentrics. These are transmitted or produce secondarily isochromosomes or fusion-centric chromosomes in the progeny F_2 investigated. The misdivision of univalents produces a break in the linkage between *Gli-D1* (1DS) and *Glu-D1* (1DL). Our results seem to demonstrate that the gametes bearing 1DS, 1DL or both, versus 1RS, 1RL or both must suffer a unbalanced competition.

Mutual dependence in pairing and co-orientation during the first meiotic division partly explains the differences in transmission of normal and alien chromosomes in double heterozygous hybrids. Thus, the presence of two translocations 1B/1BL-1RS + 1A/1AL-1RS in 'Pané' × '48-145' implies not only the formation of open bivalents involving the long arms of each pair, but also the formation of heterologous bivalents (1AL-1RS and 1BL-1RS

paired by arm 1RS), and multivalents joining up to four chromosomes. It should be noted that alternate co-orientation determines the separation of both alien chromosomes in the daughter cells. As a consequence, the occurrence of multivalents determines the preferential transmission of one over none or two alien chromosomes in the gametes of double structural heterozygotes. The mutual dependence of both pairs at segregation could condition the particular transmission or viability of one over the other. This situation could explain the different, even opposite differential rate of transmission of 1A over 1AL-1RS and 1B over 1BL-1RS in single and double heterozygotes.

The deviations detected with respect to the expected Mendelian segregation in the F_2 progenies analysed could be interpreted as a consequence of unequal transmission through the gametes, namely 'gametic selection' (assuming that only male gametes are affected). However, it might also be due to different functionality in zygotes, due to the selection of certain chromosome combinations (zygotic selection). The different response to the selection of different chromosomes or chromosome arms may be the product of an integrated contribution of meiotic behaviour plus differences in the vigour of male gametes carrying a normal chromosome compared to those carrying rye chromatin. However, it might also be due to differences in viability once male and female gametes combine.

Finally, the results appear to suggest the availability of the segregant material of the rye arm 1RS for immediate inclusion as basic material in Spanish wheat breeding programmes.

Acknowledgements: The authors would like to thank Dr J.P Gustafson for supplying the wheat-rye translocation and substitution lines, and the *Comisión Asesora de Ciencia y Tecnología* (CICYT) of Spain (Grant No. AGF97-810) for the financial support of this work.

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