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The effect of pairing regulating genes on homoeologous pairing of the three wheat genomes in wheat x rye hybrids

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ABSTRACT. The effect of pairing regulating genes located on chromosomes 5B and 3D on homoeologous pairing of the three wheat genomes is analyzed in the light of pairing at metaphase I in five wheat x rye hybrid combinations: ph1b, 5B-deficient, ph2b, 3D-deficient and normal ABDR. Only groups 1, 2, 3, and 6 with normal homoeology relationships were considered. Chromosome identification was carried out by means of C-banding. In all genotypes, preferential pairing between homoeologues of the A and D genomes occurred which indicated that the A and D genomes are more closely related than A with B or B with D. When the Ph1 gene was absent pairing between chromosomes of the B genome and their homoeologues of the A and D genomes was proportionally more increased than pairing between chromosomes of the A and D genomes. It is suggested that the Ph2 gene acts on chiasma formation. The presence of 5BS or 3DL raised the level of wheat-wheat pairing.

INTRODUCTION

Bread wheat, Triticum aestivum (2n = 6x = 42) is an allohexaploid species with the genome formula AABBDD. The 21 chromosome pairs of T. aestivum were classified into seven groups of three homoeologous chromosomes, one from each of the three genomes, by the ability to compensate for each other in nullisomic-tetrasomic combinations (Sears 1966). In spite of the genetic similarity between homoeologues, only bivalent associations of homologues are seen at diakinesis and metaphase I in wheat. Control of chromosome pairing has been attributed to several promoter and suppressor genes (for a review see Sears 1976). Ph1, located on the 5BL arm, is the most effective and acts by suppressing homoeologous pairing. Ph2, another suppressor gene located on 3DS, shows an intermediate effect. Homoeologous pairing mutants allelic to Ph1 and Ph2 were recovered after mutagenesis (Wall et al. 1971, Sears 1977, 1982, 1984).

Using a C-banding procedure, Naranjo et al. (1987, 1988) identified most wheat chromosomes and their arms in metaphase I cells of different wheat x rye hybrid (ABDR) combinations. The genotype of these hybrids (deficiency for chromosome 5B or 3D and ph1b mutant) induced homoeologous pairing. Results of pairing at metaphase I allowed to establish the arm homoeology for a majority of wheat chromosomes. Chromosomes of groups 1, 2, 3, and 6 behaved as homoeologous. The long arms of group 1, 3, and 6 chromosomes showed full pairing homoeology, as did the short arms. In group 2, the arm homoeology could not be identified. Pairing between chromosomes of homoeologous groups 4, 5, and 7 indicated the existence of normal homoeology relationships between the short and the long arms of chromosomes 4A and 4D, between 5AS, 5BS and 5DS, between 5BL and 5DL, between 7AS and 7DS, between 7AL, 7BL and 7DL, and reduced homoeology of 4BL to 7AS and 7DS, of 5AL to 4AL and 4DL, and of 7BS to 5BL and 5DL. Arms 4BL, 5AL, and 7BS are involved in a double translocation that arose during the evolution of hexaploid wheat. The homoeology realtionships of 4BS could not be identified since this arm seldom paired. The existence of a pericentric inversion in chromosome 4B of hexaploid wheat was suggested as an explanation of such a behaviour. The homoeologous pairing pattern between wheat chromosomes was characterized by a remarkable predominance of A-D associations, altered only by structural changes.

Sears (1977) following a suggestion of M. Feldman, pointed out that relative values of pairing between chromosomes of the A, B, and D genomes of wheat and chromosomes of related species would change depending on the level of pairing. Associations between chromsomes of closely related genomes would be proportionally more frequent at the intermediate level than at the high level of pairing.

Taking into account that there is

preferential A-D pairing in wheat x rye hybrids (Naranjo et al. 1987, 1988), the effect of the Ph1 and Ph2 genes and pairing promoters located on 5BS (Riley and Chapman 1967) and 3DL (Feldman and Mello-Sampayo 1967, Driscoll 1972) on the A-D, A-B, and B-D pairing types in wheat x rye hybrids is analyzed and discussed in the present work.

MATERIAL AND METHODS

Two monosomics, mono-5B and mono-3D, and two ph mutant lines, ph1b and ph2b, of Triticum aestivum cv. Chinese Spring were crossed with Secale cereale cv. Ailés. The following wheat x rye hybrids were analyzed: five ph1b ABDR plants, three 5B-deficient ABDR plants, five ph2b ABDR plants, three 3D-deficient ABDR plants, and five normal ABDR plants.

These plants were grown in a controlled environment chamber at 16-18º after vernalization for eight weeks at 6-8º. Anthers at metaphase I were fixed in 1:3 acid acetic-alcohol and were stored at 2-4º for two months as a minimum. The fixed anthers were squashed and stained according to the C-banding technique of Giraldez et al. (1979). Samples of 500 pollen mother cells (PMCs) per line were scored. In the ph1b, ph2b, and normal ABDR lines 100 PMCs per plant were analyzed; in the three ABDR-5B plants, 260, 190 and 50 PMCs; and in the three ABDR-3D plants, 280, 120 and 100 PMCs.

Wheat chromosomes were identified according to Naranjo et al. (1987, 1988).

RESULTS AND DISCUSSION

Since wheat chromosomes 4B, 5A and 7B do not show normal homoeology relationships because of structural changes (Naranjo et al. 1987, 1988), homoeologous groups 4, 5 and 7 are not considered in this work. Results of wheat-rye pairing, which was relatively low, especially at low and intermediate level of pairing, were reported by Naranjo et al (1987, 1988).

The frequency of associations at metaphase I between arms of groups 1, 2, 3, and 6 chromosomes are given in Table 1. Triple chromosome arm associations ABD were found in configurations such as Y-shaped trivalents, frying pan trivalents, bird cage trivalents, and some quadrivalent and quinquevalent types. The

Table 1. Frequency (%) of associations at metaphase I between homoeologous chromosome arms of wheat belonging to groups 1, 2, 3, and 6 in five different wheat x rye hybrid genotypes.

Group and ABDR genotype	Long arm associations				Short arm associations			
	AD	BD	AB	ABD	AD	BD	AB	ABD
Group 1								
ph1b[a]	12.00	59.00	7.00	17.00	39.00	3.00	2.00	-
ph1b	71.25	10.00	7.50	5.75	40.00	7.00	4.00	0.25
5B-deficient	59.00	8.20	8.40	5.60	14.80	2.20	1.80	0.60
ph2b	65.20	3.40	3.80	0.60	13.80	1.40	0.80	0.60
3D-deficient	35.00	2.00	1.40	-	4.60	0.80	0.20	-
normal	14.80	1.00	0.80	-	1.20	0.20	-	-
Group 2[b]								
ph1b	46.60	47.60		15.80	46.60	22.60		3.60
5B-deficient	37.50	43.00		15.40	37.50	18.20		2.60
ph2b	34.00	28.60		3.00	34.00	6.20		0.20
3D-deficient	15.40	16.40		-	15.40	1.80		-
normal	5.90	6.80		-	5.90	0.20		-
Group 3								
ph1b[a]	92.33	2.33	2.00	2.00	63.67	11.33	4.00	2.00
ph1b	54.00	16.00	7.00	11.00	54.50	11.00	6.00	2.00
5B-deficient	41.40	17.00	10.00	5.60	27.80	10.20	3.80	1.20
ph2b	31.80	9.80	3.20	1.40	19.00	1.40	0.60	-
3D-deficient			4.00				1.00	
normal	6.00	1.80	0.60	-	0.80	0.40	-	-
Group 6								
ph1b	80.20	7.80	3.40	3.20	79.80	4.60	4.40	1.20
5B-deficient	60.60	11.80	6.80	4.00	59.60	2.60	1.60	0.60
ph2b	54.00	3.40	0.80	0.40	33.40	1.40	0.80	-
3D-deficient	24.40	0.60	0.40	0.20	11.00	0.60	-	-
normal	8.20	0.60	-	-	3.00	0.40	-	-

Note. Dashes indicate that no observations were made. A total of 500 PMCs per line were scored.

a: Plants with translocations involving homoeologous chromosomes: one plant (100 PMCs) in group 1 and three plants (300 PMCs) in group 3.

b: Neither chromosomes 2A nor 2D, nor their arms, were identified. AB and BD associations were pooled; the value of the AD associations s the mean for the short and the long arms.

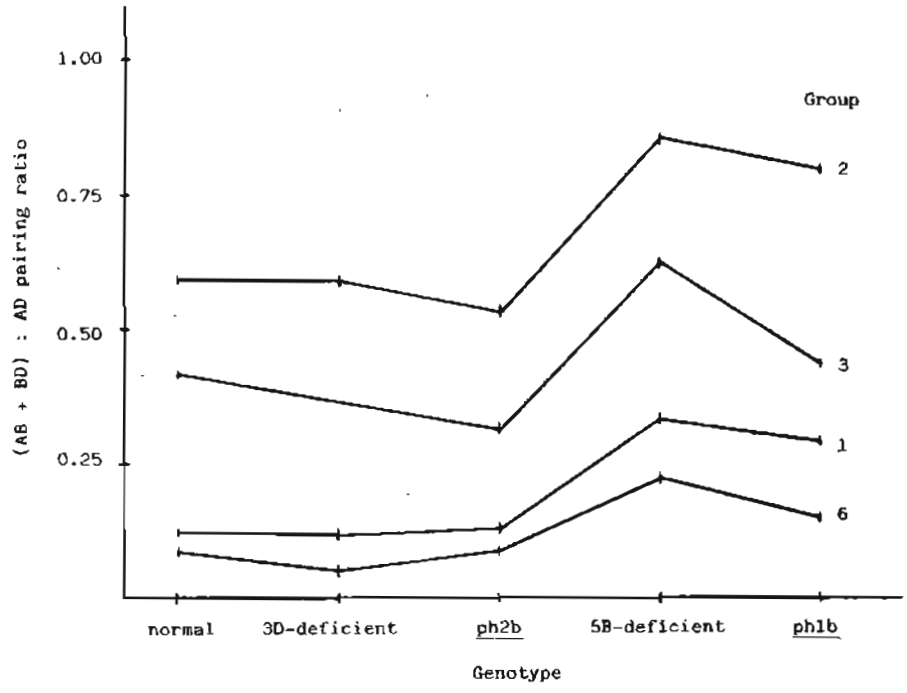

Figure 1. Variation of the (AB + BD) : AD pairing ratio with the genotype for pairing regulating genes in homoeologous groups 1, 2, 3, and 6 in wheat x rye hybrids.

A-D pairing type was much more frequent than pairing involving chromosomes of the B genome in all genotypes. This result indicates that pairing regulating genes do not qualitatively change the pattern of pairing and, therefore, the A and D genomes are more closely related than A with B or D with B.

Deviation from the standard pairing pattern appeared in group 1 chromosomes in one ph1b hybrid, and, in group 3, in three other plants (Naranjo et al. 1988). It was concluded that, in the first plant, 1BL had a terminal segment from 1DL replacing the corresponding 1BL segment; the three other plants carried in duplicate a terminal segment either from 3AL or 3DL and simultaneously a deficiency for its homoeologous segments in 3DL or 3AL, respectively. Such strucutural changes arose as a result of homoeologous recombination in the ph1b mutant wheat.

In order to check whether the rate of pairing involving B-genome chromosomes (A-B and B-D pairing types) varied depending on the level of pairing, the (AB + BD) : AD pairing ratio was calculted in all genotypes. Results are shown in Figure 1. The long and the short arms were pooled in all homoeologous groups. In groups 1 and 3, values of ph1b hybrids were obtained from plants with normal chromsosome structure. Since, in triple chromosome arm associations ABD, the actual pairing could not be determined, it was considered that such associations consist of a bond between the arms of the A and D genomes and another one of the A-B or B-D type. Pairing of B-genome chromosomes could be underestimated because, in some ABD arm associations, the chromosome arm of the B genome could be paired with the other two arms.

The (AB + BD) : AD pairing ratio showed similar values at low (normal hybrids) and intermediate level of pairing (ph2b and 3D-deficient hybrids) but increased at high level of pairing, especially in the 5B-deficient genotype. The increase was apparent in the four homoeologous groups. From this result the following conclusion can be drawn: the Ph1 gene suppresses more efficiently homoeologous pairing of chromosomes of the B genome, that is, pairing between more differentiated chromosomes. This conclusion supports, at least in part, the Sears' (1982) suggestion that ph2b mutation may suffice to induce the transfer of genes to wheat from alien chromosomes closely related to one of the three wheat genomes. If alien chromosmes belong to species closely related to the B genome of wheat the transfer of genes may be achieved using the ph2b mutation. In the case that alien chromosomes were more closely related to either the A genome or the D genome than to the B genome, competitive A-D pairing would probably hinder pairing between the alien chromosomes and wheat chromosomes in the ph2b genotype.

Several hypotheses about the mode of action of the Ph1 gene have been proposed. Feldman and Avivi (Feldman 1966, Feldman and Avivi 1984, 1988) suggested that Ph1 exerts its effect during premeiotic stages, before the commencement of synapsis, thereby controlling chromosomal spatial relationships. In euploid wheat, two copies of Ph1 would ensure a close

presynaptic association of homologues, homoeologues staying separated, wich results in a exclusive presence of homologous bivalents at metaphase I. In the absence of Ph1 (nully-5B or ph1 mutant) both homologous and homoeologous chromosomes would be closely associated at presynaptic stages. This results at meiosis in homologous and homoeologous pairing and in interlocking of homoeologous bivalents that fail to form a multivalent. In plants triisosomic for 5BL (i. e. with six doses of Ph1) neither homologous nor homoeologous are associated at premeiosis. Meiotic pairing becomes more random resulting in partial asynapsis of homologous chromosomes, in homoeologous pairing, and in interlocking of non-homoeologous bivalents as a result of pairing between slightly separated partners. Feldman and coworkers (Avivi et al 1982, Yacobi et al. 1985a and b) have reported evidence that the three wheat genomes tend to ocupy different areas in somatic and meiotic nucleus. In a study of the position of marked bivalents formed by a telocentric and a complete chromosome, Heslop-Harrison et al. (1985) concluded that bivalents from the B genome tend to lye more often at the outside of the plate than bivalents from the A and D genomes. They suggested that, in hexaploid wheat, the three genomes might be concentrically separated with the outer, peripheral, B genome tending to sourround the more central A and D genomes.

Hobolth (1981) analyzed meiotic pairing in hexaploid wheat by complete three dimensional reconstruction of a late zygotene and an early pachytene nucleus. At zygotene, multivalent configurations appeared while, at pachytene, pairing was exclusively in bivalents. Hobolth proposed that the role of the Ph1 gene is to delay crossing over untill the pairing correction is completed at early pachytene. In the absence of Ph1 crossing over could occur when correction of multivalent pairing is not yet completed, and multivalents persist to metaphase I as a consequence of chiasma formation between homoeologous chromosomes. Six doses of Ph1 would delay crossing over so long that chiasma frequency decreases.

Gillies (1987) studied chromosome pairing at zygotene-pachytene in T. aestivum x T. Kotschyi hybrids carrying either the Ph1 allele or the ph1 allele by electron microscopy of synaptonemal complexes in spread microsporocyte nuclei. He concluded that the Ph1 control on homoeologous pairing does not act on the ability to pair into synaptonemal complexes but affects the ability to generate crossover.

Holm and coworkers (Holm and Wang 1988 and references therein) have recently reported a thorough study about the effect of the Ph1 gene on synapsis and chiasma formation in hexaploid wheat. They have analyzed spread mycrosporocyte nuclei from euploid, mono-5B, nulli-5B, monoiso-5BL, diiso-5BL, and triiso-5BL plants of Chiness Spring; from trihaploid wheat (ABD) with and without chromosome 5B; and from wheat x rye hybrids (ABDR) with 0, 1, or 2 copies of the 5BL arm. Their results disagree with the Feldman's predictions. The analysis of the leptotene-zygotene transition stage do not provide any indication of the existence of a large scale alignment of homologues in euploid and mono-5B wheat. Short and unspecific alignment between many different combination of lateral components occurs at the initiation of synapsis. On the other hand, haploids with chromosome 5B showed estensive synaptonemal complex formation. In nully-5B, monoiso-5BL, diiso-5BL and triiso-5BL wheat, synapsis is incomplete. The extent of synapsis relates to the interlocking frequency at metaphase I. On the basis that in organisms where the synapsis is complete all interlockings are usually resolved, Holm and Wang (1988) suggest that when synapsis is arrested before completion several interlockings are not recognized and resolved, and persist untill metaphase I.

Likewise, Holm and Wang (1988) disprove the hypothesis of Hobolth (1981) by the following: The decrease in chiasma frequency in triiso-5BL in relation to euploid wheat is explained by pairing arrest at early zygotene. In euploid and mono-5B wheat, some multivalent persist at pachytene but no multivalent configuration is observed at metaphase I. In haploid wheat carrying chromosome 5B, pairing configurations are not corrected into univalents before the degradation of synaptonemal complexes is intiated. On the other hand, Holm amd Wang (1988) found that the absence of chromosome 5B increases the number of lateral components in multiple associations already from the begening of zygotene in hexaploid wheat, and the number of pairing partner exchanges per lateral component in trihaploid wheat and wheat x rye hybrids. Only in euploid wheat are the pairing partner exhanges almost completely corrected at pachytene. Holm and Wang (1988) propose that the product of the Ph1 gene affects the stringency of synapsis as well as the suppression of crossing over between partially homologous chromosomes.

The relatively higher increase of associations involving B-genome chromosomes in absence of Ph1 (deficiency for chromosome 5B or presence of the ph1b allele) in wheat x rye hybrids (Table 1 and Figure 1) is in agreement with the proposal of Holm and Wang (1988). Among the three genomes of wheat, the B genome is the most differentiated and, therefore, the most affected one by the activity of the Ph1 locus. In hybris without the Ph1 allele, the recognition process between lateral components would be less stringent; consquently, chromosomes of the B-genome would be permitted to align and form synaptonemal complexes with their homoeologues more frequently than in presence of Ph1. The increase in the number of pairing partner exchanges per lateral component produced by the absence of chromosome 5B in wheat x rye hybrids (Wang and Holm 1988) might be the result of a more active participation of B-genome lateral components on synaptonemal complex

formation.

The (AB + BD) : AD pairing ratio has slightly lower values in ph1b hybrids than in 5B-deficient hybrids (Figure 1). This decrease was most likely due to the presence of the 5BS arm in the ph1b hybrids. Chromosome arm 5BS increases the level of associations between homoeologues at metaphase I in wheat x rye hybrids (Table 1, Naranjo et al. 1988). Holm and Wang (1988) indicate that the absence of the 5BS arm brings about incomplete synapsis in hexaploid wheat. It is possible that in ph1b hybrids the extent of synapsis was higher than in 5B-deficient hybrids, and that A-D pairing elongated more than A-B or B-D pairing. Thus, the number of chiasmata generated in A-D associations could increase more than that of A-B or B-D pairing.

The presence or absence of the Ph2 gene does not significantly modify the (AB + BD) : AD metaphase I pairing ratio in wheat x rye hybrids (Figure 1). Nevertheless, the frequency of arms being bound at metaphase I is higher in absence (3D-deficient and ph2b ABDR genotypes) than in presence of Ph2 (normal hybrids) (Table 1). Under the assumption that synaptonemal complexes formed in trihaploid wheat and wheat x rye hybrids carrying this gene (Holm and Wang 1988) involved homoeologous chromosomes, the product of the Ph2 gene would act suppressing crossing over between such homoeologous chromosomes. By a similar reasoning the promoter activity of 3DL would also influence chiasma frequency.

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