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Meiotic pairing and alpha-amylase phenotype in a 5B/5R^m *Triticum aestivum – Secale montanum* translocation line in common wheat

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Summary. A 5BS/5R^{m}S translocation chromosome spontaneously recovered from a 'Chinese Spring'–*Secale montanum* wheat-rye telocentric 5R^{m}S addition line has been identified and cytologically studied using C-banding in somatic and meiotic cells. Analysis of the translocated chromosome showed that a terminal segment of the short arm of 5B had been replaced by a short terminal region of chromosome arm 5R^{m}S. The translocation led to the deletion of the genetic system promoting pairing located in 5BS, which is slightly compensated for when doses of 5R^{m}S are increased, indicating homoeology to wheat chromosome 5BS. The α-amylase phenotype in 5B/5R^m translocated material was studied and found to be identical to that of ditelocentric line 5BL of 'Chinese Spring'. An effect on the α-amylase activity was detected as a result of the removal of the terminal region of 5BS, perhaps as a consequence of variation in dormancy period duration.

Key words: α-amylase – *Triticum aestivum* – *Secale montanum* – C-banding – Meiotic pairing

Introduction

The addition of alien chromosomes obtained from various diploid relative species to the hexaploid wheat (*Triticum aestivum* L. em Thell 2n=6x=42) genome is becoming a good procedure to get information on genetic relationships in the Triticeae and for the introduction of new agronomic characters into that important crop.

Translocations between wheat and alien chromosomes often occur in the wheat-alien addition lines and can be of interest because the amount of the alien genetic material is decreased reducing the chance of introducing undesirable characteristics into wheat. Moreover, wheat-alien translocations may also lead to the substitution of wheat genes or chromosomal regions and cause interesting interactions between wheat and alien gene products. Translocations affecting wheat chromosomes carrying genes that control meiotic chromosome pairing can add new information to the knowledge of the cytological effect of that genetic system. The pairing of homoeologous chromosomes of wheat is mainly prevented by the activity of the *Ph* suppressor gene which is located in the long arm of chromosome 5B (Okamoto 1957; Sears and Okamoto 1958; Riley and Chapman 1958; Riley 1960; Wall et al. 1971).

The present paper deals with the characterization and study of a 5B/5R^m translocation line spontaneously obtained from a *T. aestivum* cv. 'Chinese Spring' – *Secale montanum* 5R^{m}S addition line (2n=6x=42+2 telo). The cytological analysis used C-banding. Both the meiotic behaviour at first metaphase and the α-amylase zymogram phenotypes are involved in the present study.

Materials and methods

Materials analysed in the present work consisted of the following materials: *Secale montanum* Guss. (2n=14; genome R^{m}R^m); *Triticum aestivum* L. cv. 'Chinese Spring' (2n=6x=42; genomes AABBDD); cv. 'Chinese Spring' ditelocentric 5BL (2n=6x=40+2 telo 5BL); cv. 'Chinese Spring' structurally homozygous for the translocation 5B/5R^{m}S (2n=6x=42) (T5B/5R^m); cv. 'Chinese Spring' translocated and monotelosomic for the addition of one dose of the 5R^m short arm (2n=6x=42+1 telo 5R^{m}S) (T5B/5R^m+t5R^{m}S) cv. 'Chinese Spring' translocated and ditelosomic for the addition of 5R^m short arm (2n=6x=42+2 telo 5R^{m}S) (T5B/5R^m+2t5R^{m}S).

The 5B/5R^m translocation appeared spontaneously in the progeny of *T. aestivum* cv. 'Chinese Spring' telocentric 5R^{m}S addition line. This aneuploid line was kindly supplied by T. E. Miller from the Department of Cytogenetics PBI, Cambridge,

England. The translocation was discovered while attempting to study the meiotic behaviour of that addition line.

Somatic cells at metaphase from root meristems were karyotypically analysed using the Giemsa C-banding technique reported previously (Jouve et al. 1980). The 5B, $5R^m$ and $5BS/5R^mS$ chromosomes were identified by their C-banding pattern in both somatic (described in 'Chinese Spring' by Gill and Kimber 1974; Van Niererk and Pienaar 1983) and meiotic (Ferrer et al. 1984a) cells. The karyotype of *Secale montanum* was also studied using C-banding. Our chromosome identification is based on the C-banded chromosome descriptions of Gustafson et al. (1976).

Heterochromatin staining along with chromosome morphology and densitometric analysis were used to identify the 5B and $5R^m$ chromosomes respectively in 'Chinese Spring' and *S. montanum*. The analysis was also extended to the 'Chinese Spring' – *S. montanum* translocated plants in order to detect cytologically translocational segments.

Anthers from plants studied were fixed in 1:3 acetic-alcohol; meiotic observations were made in at least 100 pollen mother cells (PMCs) at first metaphase in Giemsa stained preparations.

For the α-amylase analysis 5 day-old germinated grains, maintained at 21 °C in a growth chamber with 15.5 h daylight photoperiod, were used. Individual samples of endosperm were immersed in 75 µl of 0.05 M tris-ClH buffer (pH 8.6). The half grains were homogenised and incubated at 60 °C for 15 min. The method described by Nishikawa and Nobuhara (1971) was used with minor modifications. Small pieces of filter paper (Whatman 3 MM, 2×10 mm) were soaked in the extract and then inserted into the polyacrilamide gels (180×280×2 mm) (8% w/v). The gel and electrode buffer was 0.2 M Tris-glycine (pH 8.7). The samples were migrated to the anode at room temperature at 120 V during 16 h. The gels were immersed in a 1% (w/v) starch solution in 0.4 M phosphate buffer (pH 7.0) for 2 h at 37 °C and stained with an iodine solution as described by Sargeant and Walker (1978).

Results and discussion

Characterization of $5BS/5R^mS$ translocation

Preliminary studies at the cytological level in PMCs allowed recognition of the existence of an important modification in the C-banding pattern of the 5B short arm in plants of a sample of the *T. aestivum* cv. 'Chinese Spring' – *S. montanum* telo $5R^mS$ addition line ($2n=6x=42+2$ telo). The 5B submetacentric chromosome commonly shows an intensely stained region around the centromere which is mainly extended towards the short arm. Two intercalary bands in the long arm and one faint subtelomeric band in the short arm are also observed.

In some plants coming from the above mentioned source, the 5B chromosome exhibited a large telomeric dark band in its short arm (Fig. 1). As the plants in which this modification was being observed were derived from a *T. aestivum* – *S. montanum* $5R^mS$ addition line, the existence of a $5B/5R^m$ translocation was inferred.

Analysis of the somatic chromosomes in 'Chinese Spring', both normal and modified, and in *S. montanum* was carried out. Chromosome $5R^m$ exhibits a dark telomeric and one faint subtelomeric band in the short arm. Figure 2 shows the results of the application of C-banding and densitometry when comparing the heterochromatic pattern in the 5B, $5R^m$ and $5B/5R^m$ chromosomes.

The large heterochromatic block observed in the telomeric region of the 5B chromosome seems to be identical to that of the short arm in the $5R^m$ chromosome. The breakage point may be located between the pericentromeric and subtelomeric bands of the 5B short arm, respectively present and absent in the translocated chromosome. The terminal region of $5R^mS$ does not include the faint intercalary band.

Translocations involving rye and wheat chromosome segments of the same homoeologous group have been previously reported. Mettin et al. (1973), Zeller (1973) and Shepherd (1973) have described translocations involving rye chromosome arm 1RS and wheat chromosome segments of homoeologous group 1 (long arms). Another translocation included segments of 2AL and 2RL (Sears 1972) and revealed a significant increase in kernel protein content (May and Appels 1978). Acosta (1961) produced a 3A/3R wheat-rye translocation which was cytologically analyzed by Barber et al. (1968). The long arm of rye chromosome 5R, carrying the hairy neck gene, was described as translocated into wheat chromosomes 4A (Driscoll and Sears 1965), 5BS (Sears 1967), 6BL (Sears 1973), 5DL (Muramutsu 1969) and 6D (Sears 1967). Two wheat-rye chromosome translocations, 4A/7R and 7B/4R, were spontaneously produced in the progeny of *T. aestivum* cv. 'Chinese Spring' – *S. cereale* cv. 'Imperial' rye substitution lines 4R and 7R (Zeller and Koller 1981). Finally, a short segment of rye chromosome 2R was detected in one line of common wheat derived from the backcross (*T. aestivum*×*S. cereale*)×*T. aestivum* by Fominaya et al. (1985). The pairing and crossing-over of an alien chromosome with one of its wheat homoeologues has been proposed as an excellent way of accomplishing the substitution of an alien segment for a closely related wheat segment.

However, wheat-rye homoeologous pairing is restricted to a very low level (Riley 1960; Bieling and Driscoll 1970; Dhaliwal et al. 1977). Hutchinson et al. (1983), using C-banding and a 5B defficient hybrid, showed that wheat and rye chromosomes pair together rather infrequently.

Chromosome pairing

The distribution of chiasmata frequencies per PMC observed in the plants analysed are shown in Fig. 3. Riley and Chapman (1967) demonstrated the existence of a gene on the short arm of chromosome 5B which has an effect opposite to that of *Ph*-namely it promotes pairing. In our material the absence of arm 5BS (ditelo-5BL) results in a decreased level of pairing. The absence of a terminal segment in the short arm of chromosome 5B ($5BS/5R^mS$ translocation) results in no more pairing than complete deficiency for 5BS. Thus, the $5B/5R^mS$ translocation results in a level of pairing

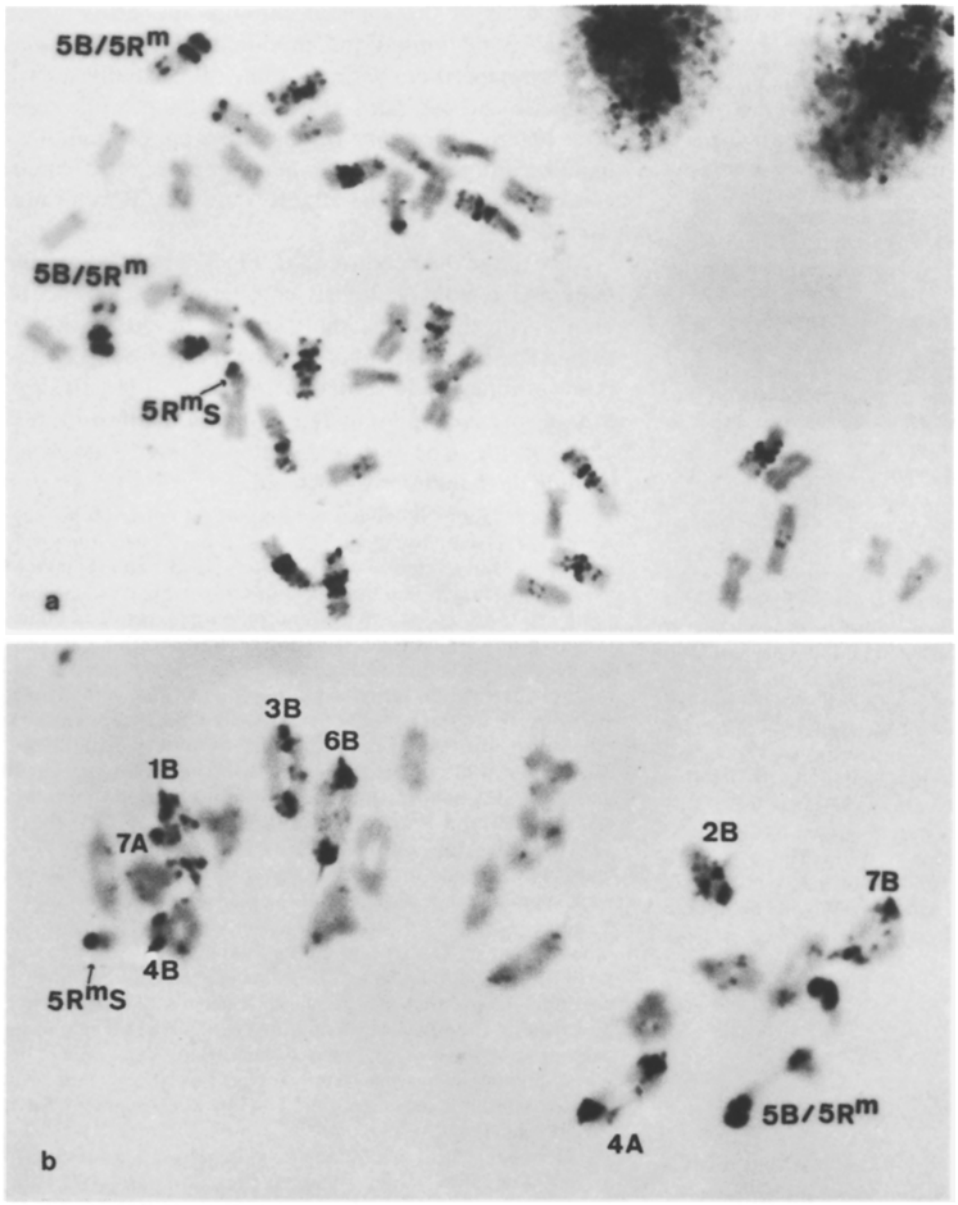

Fig. 1. Photomicrographs showing **a)** somatic chromosomes of the 5B/5R^m translocated plants (having the addition of a 5R^m short arm. **b)** meiotic pollen mother cell at metaphase I exhibiting the 5B/5R^{m}S pair as an open bivalent

as low as that caused by the deficiency for 5BS. Finally, the addition of successive doses of chromosome arm 5R^{m}S to the chromosome complement of 5B/5R^m translocated material leads to an increase of pairing.

There seems to be an interaction between genetic systems which affects pairing located in the 5R^{m}S and 5BL chromosome arms. The total mean number of chiasmata per PMC was successively enhanced when passing from two (42.5 ± 3.2) to three (46.0 ± 3.4) and from three to four (47.4 ± 3.9) doses of either the terminal segment or total 5R^{m}S arm.

The short arm 5R^{m}S of *S. montanum* seems to carry a genetic system that slightly promotes meiotic pairing, compensating for the deficiency of chromosome arm 5BS. As a consequence, homoeology between the short arms of 5R^m and 5B can be assumed.

The C-banding method permitted the identification of nine chromosome pairs in all genotypes (Fig. 2). Individual frequencies of bound arms were scored on the basis of the number of chiasmata per arm in each chromosome. The results are given in Table 1. In agreement with results found in earlier works (Sallee and

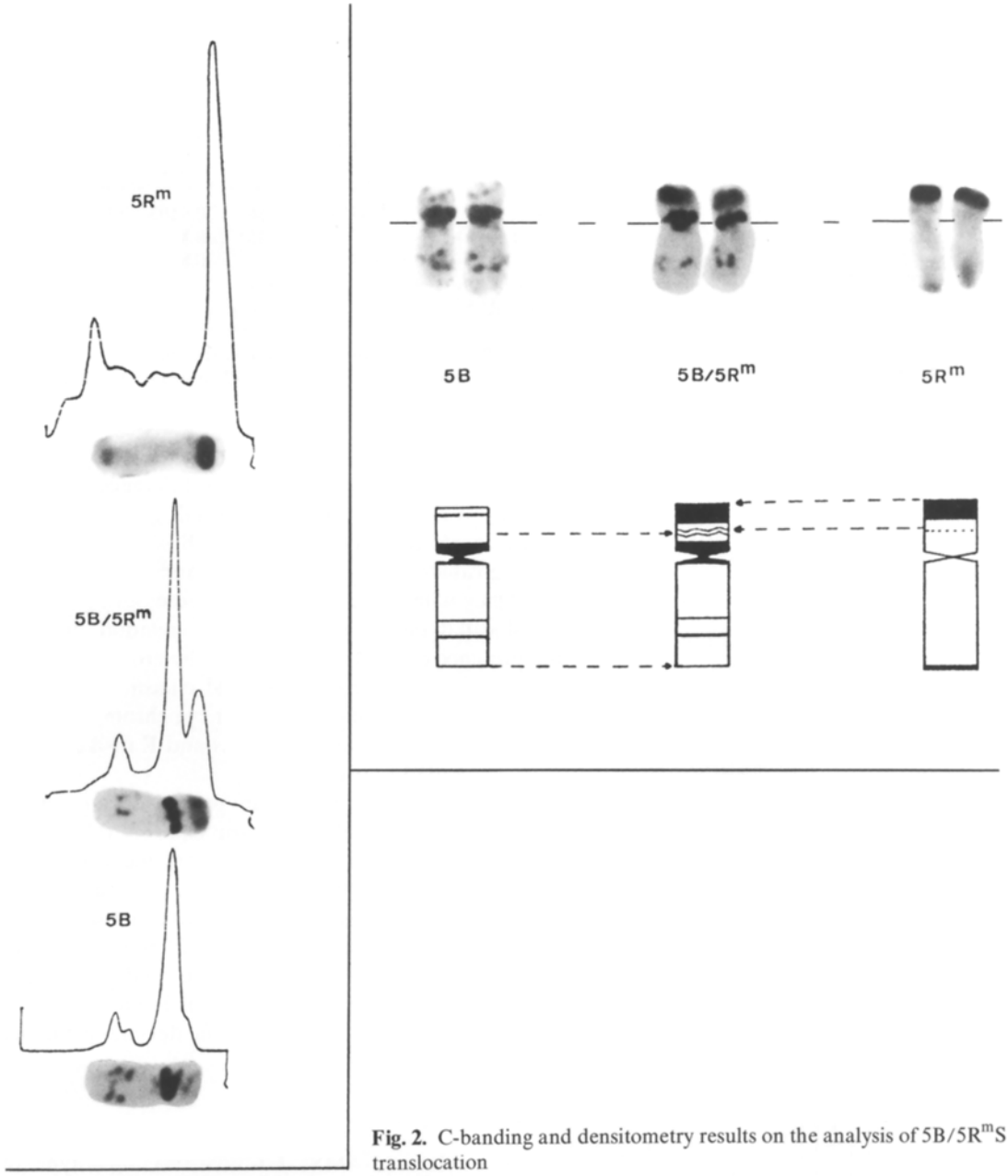

Fig. 2. C-banding and densitometry results on the analysis of 5B/5R^{m}S translocation

Table 1. Mean pairing arm-to arm frequencies for each genotype

Plant material	Arm	1B	2B	3B	4B	5B	6B	7B	4A	7A	Xata/arm in others chromosomes
CS	L	1.26	1.22	1.34	1.47	1.66	1.11	1.32	0.96	0.99	1.11
	S	0.98	1.13	1.01	0.79	0.67	1.12	0.79	0.79	1.18	
dt5BL	L	1.18	1.11	1.09	1.38	1.52	1.09	1.33	0.99	1.13	1.00
	S	0.92	0.94	0.97	0.79	–	0.95	0.83	0.96	0.96	
T5B/5R^m	L	1.20	1.04	1.09	1.50	1.57	1.00	1.35	0.97	0.88	1.02
	S	0.84	1.07	0.96	0.70	0.35	0.88	0.90	0.76	1.12	
T5B/5R^m	L	1.29	1.42	1.49	1.61	1.66	1.12	1.29	1.15	1.20	1.06
+t5R^{m}5	S	1.00	1.22	1.03	0.92	0.53	1.07	0.91	0.87	1.00	
T5B/5R^m	L	1.25	1.25	1.37	1.71	1.78	1.40	1.53	0.96	1.03	1.11
+dt5R^{m}5	S	0.90	0.96	1.16	0.75	0.43	1.25	0.96	0.71	1.21	

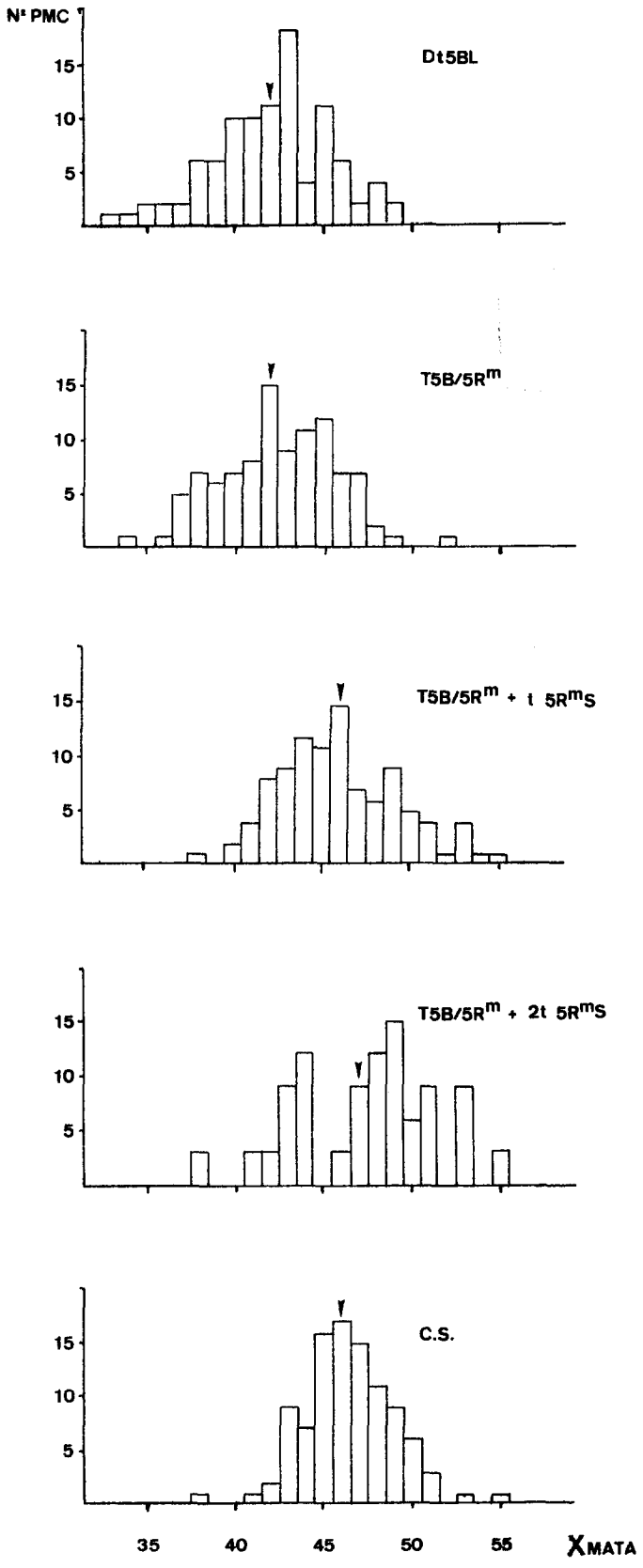

Fig. 3. Frequency distribution of chiasmata in pollen mother cells (PMC) in each genotype. *Arrows* represent means

Table 2. Correlation coefficients between data series on arm pairing between genotypes

	CS	dt5BL	T5B/5R^m	T5B/5R^m +t5R^{m}S	T5B/5R^m +dt5R^{m}S
CS		0.8757[a]	0.9266	0.9193	0.9346
dt5BL	+++		0.8945[a]	0.8584[a]	0.8692[a]
T5B/5R^m	+++	+++		0.8886	0.9253
T5B/5R^m +t5R^{m}5	+++	+++	+++		0.8702

+++ Positive and high significant correlations
[a] Correlation coefficients based on 17 chromosome arms

Kimber 1978; Ferrer et al. 1984b) differences of arm-to-arm pairing are observed in all genotypes. The translocated chromosome 5B/5R^{m}S showed normal pairing values in its long arm (5BL) and a lower pairing frequency with respect to 5BS ('Chinese Spring') in the translocated one (5BS/5R^{m}S). The relation between the presence of a telomeric block of heterochromatin in the 5R^{m}S translocated region and chiasma formation has been largely demonstrated for rye chromosomes in *S. cereale* and triticale (Roupakias and Kaltsikes 1977; Kaltsikes and Gustafson 1985).

Between plant variation in pairing was analysed comparing data sets of arm-to-arm chiasma values. The correlation coefficients estimated from mean frequencies of pairing per chromosome arm had values of r near to 0.9 in all cases and were positive and significant (Table 2). It can be concluded that differences in genes affecting pairing in the plants here studied affected chiasmata levels in chromosome arms in an indiscriminate way, and did not lead to specific deviations for any of them.

α-amylase isozymes

The α-amylase (α-AMY; E.C. 3.2.1.1) phenotype of euploid 'Chinese Spring' consists of 10 distinct bands. The absence of arm 5BS in ditelo5BL or its terminal segment in 5B/5R^{m}S causes the addition of band 9 (Fig. 4, lanes 1 and 4) and the weakness of band 3 relative to the euploid.

These results seem to indicate that bands 3 and 9 are dependent on the lack of expression of genes located on the terminal region of arm 5BS. Although band 3 does not completely disappear upon the removal of the critical region on arm 5BS, its relative staining intensity is remarkably reduced relative to the euploid. However, both ditelo5BL and translocated 5B/5R^{m}S genotypes possessed an identical phenotype, indicating that the decrease in relative staining intensity of band 3 is not due to the presence of the 5R^m segment but to the loss of a short terminal region in 5BS.

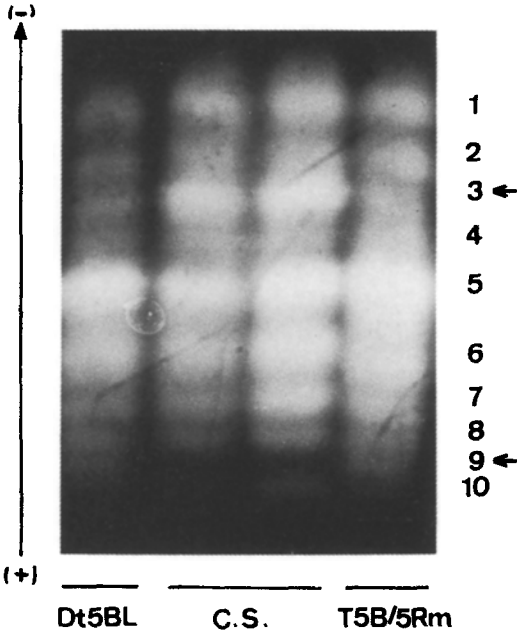

Fig. 4. α-amylase observed in each genotype. *Arrows* show the differences in the zymograms

An opposite effect is observed in the expression of band 9, which is present in the zymogram only in the absence of the 5BS terminal segment.

The α-amylase isozymes are controlled by genes which have been located on the homoeologous groups 6 and 7 of wheat (Nishikawa and Nobuhara 1971; Hart 1979; Gale et al. 1983). Moreover, our results suggest the location of genes affecting α-amylase activity during germination to be on the terminal region of chromosome arm 5BS. α-amylase activity is perhaps affected by a variation in the post-dormancy period duration. This assumption is in agreement with the results of Gale et al. (1981) who indicated, by studying reciprocal chromosome substitution lines, that the genetic control of the duration of dormancy and its variation in α-amylase activity on germination could be attributed predominantly to chromosomes of homoeologous groups 5 and 7.

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