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Nucleolar competition in different (A/B)(A/B)RR and DDRR tetraploid triticales

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Nucleolar activity was analysed in hybrids between *Aegilops squarrosa* and *Secale cereale* or *Secale silvestre* and in different tetraploid triticales with A/B mixed genomes, using phase contrast, C-banding and Ag-NOR staining techniques. The results revealed that nucleolar activity of the satellite chromosome pair 1R of *S. cereale* as well as that of *S. silvestre* was suppressed by the presence of the nucleolus organising chromosome of *Aegilops squarrosa*. In all combinations of tetraploid triticales with A/B mixed genomes containing satellite chromosome pairs 1B and 6B or only chromosome pair 1B, nucleolar activity was restricted to satellite chromosomes from the B genome while those belonging to the R genome of rye and A genome of wheat were completely suppressed. Satellite chromosome pair 1R of rye was active in producing nucleoli, only in combinations missing 1B and 6B while nucleolar organizer regions from A genome remained inactive.

INTRODUCTION

Ribosomal DNA contains 18S and 28S repeated sequences tandemly disposed. The chromosomal sites of these rRNA genes are known to be located in nucleolar organiser regions (NORs). Previous studies revealed the existence of a very close correlation between transcriptional activity for rRNA genes and the presence of a secondary constriction at NORs of satellited chromosomes (Bush and Swetanak, 1970; Flavell and O'Dell, 1976; Martini and Flavell, 1985).

Recently a silver staining technique has been developed in order to analyse nucleolar activity by means of conventional light microscopy, considering that a positive Ag-NOR reaction at mitotic metaphase reflects genetic activity at the preceding interphase (Goodpasture and Bloom, 1975).

Differential nucleolar activity has been reported in many species and interspecific hybrids using conventional staining procedures (Howell, 1982), but in many of these cases the identification of the critical chromosomes was not possible. By using differential staining techniques nucleolar activity of specific chromosomes has been studied in more detail. In hexaploid wheat the satellite chromosome pairs 1B and 6B are responsible for the major production of nucleoli, while chromosome pair 5D exhibits only minor nucleolar activity (Thomas and Kaltsikes, 1983; Martini and Flavell, 1985; Viegas and Mello-Sampayo, 1975). NORs from the A genome of wheat are suppressed completely. A similar suppression of nucleolar organiser chromosome pair 1R from *Secale cereale* has been detected in hybrids between wheat and rye (Cermeño *et al.*, 1984; Lacadena *et al.*, 1984).

However, since so far in the triticales analysed complete A, B and D genomes of wheat were always present, nucleolar activity could not be studied in a more specific way. Different lines of tetraploid triticales (A/B)(A/B)RR with A/B mixed genomes of wheat offer the opportunity to analyse nucleolar activity in different combinations of diploid rye (RR) with A and B or D genomes of wheat.

MATERIAL AND METHODS

The following (A/B) and D genomes of wheat with R^c from *Secale cereale* and R^s from *Secale silvestre* were analysed:

(a) *Aegilops squarrosa-Secale silvestre* hybrid ($2n = 28$), genomic constitution DDR^sR^s, and *Aegilops squarrosa-Secale cereale* hybrid ($2n = 28$), genomic constitution DDR^cR^c;
(b) Different combinations of *Triticum turgidum* and *T. durum-Secale cereale* ($2n = 28$), genomic constitution
(1B1B, -, -, -, -, 6B6B, -)R^cR^c
(1B1B, -, -, -, -, 6A6A, -)R^cR^c
(1A1A, -, -, -, -, 6A6A, -)R^cR^c

Table 1 Silver-stained nucleolar organiser regions (Ag-NORs) and nucleoli visualised in somatic metaphase and interphase cells in different tetraploid wheat–rye combinations

Combination	No. of plants	Meta-phases	Ag-NORs	No. of nucleoli 1	2	3	4	Total cells	χ^2	d.f.	p
DDR^sR^s	3	15	2	576	407	—	—	983	0.44	2	$0.90 > p > 0.75$
DDR^cR^c	4	14	2	1035	740	—	—	1775	4.00	3	$0.50 > p > 0.25$
(1B1B, -, -, -, -, 6B6B, -)R^cR^c	24	54 19	4 3	1707	2690	963	100	5460	17.23	23	$p > 0.99$
(1B1B, -, -, -, -, 6A6A, -)R^cR^c	11	39	2	1254	927	—	—	2181	9.43	10	$0.50 > p > 0.25$
(1A1A, -, -, -, -, 6A6A, -)R^cR^c	4	25	2	1057	845	—	—	1902	7.52	3	$0.50 > p > 0.25$

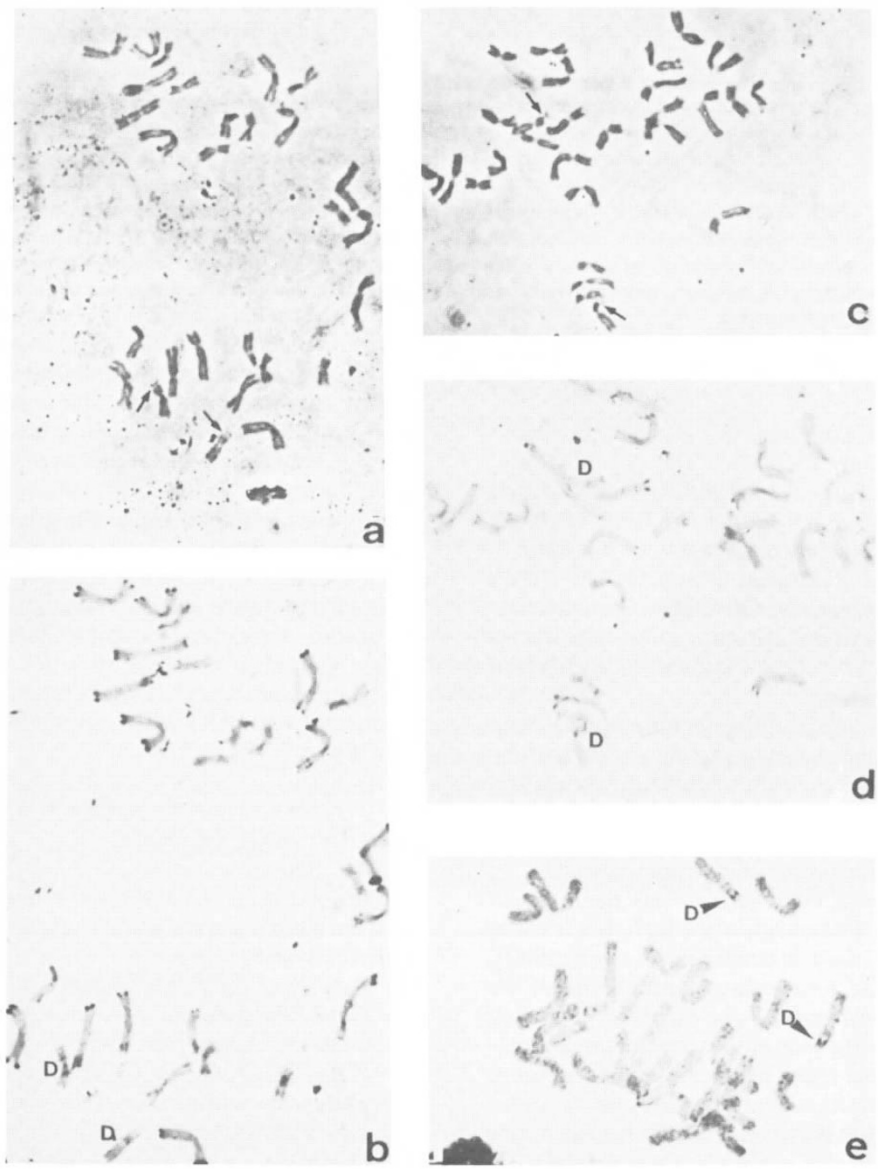

Figure 1 Phase contrast, C-banding and Ag-NOR-banding of somatic metaphase cells of *Aegilops squarrosa*-rye tetraploid triticales. (a), (b) and (e), *A. squarrosa–S. cereale*, phase contrast, C-banding and Ag-NOR-banding. (c) and (d), *A. squarrosa–S. silvestre*, phase contrast and C-banding. Arrows indicate secondary constrictions and Ag-NOR bands.

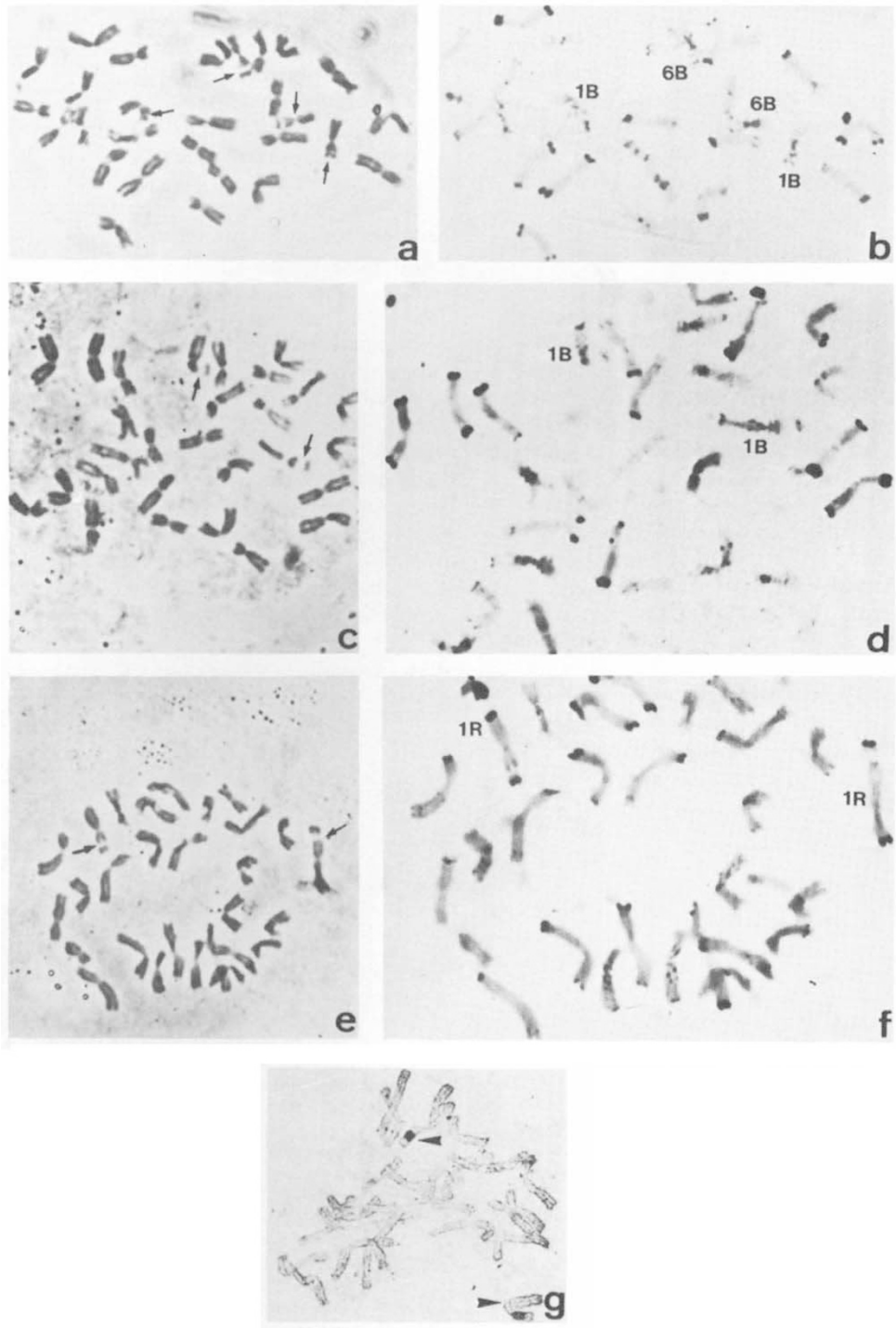

Figure 2 Phase contrast, C-banding and Ag-NOR-banding of somatic metaphase cells of tetraploid triticales with A/B mixed genomes. (a) and (b), (1B1B, -, -, -, -, 6B6B, -)R^cR^c, (c) and (d), (1B1B, -, -, -, -, 6A6A, -)R^cR^c and (e) and (f), (1A1A, -, -, -, -, 6A6A, -)R^cR^c, phase contrast and C-banding. (g), (1A1A, -, -, -, -, 6A6A, -)R^cR^c, Ag-NOR-banding. Arrows indicate secondary constrictions and Ag-NOR bands.

A comparative analysis of somatic metaphase cells was carried out by means of phase contrast followed by C-banding or Ag-NOR staining according to Giraldez *et al.* (1979) and Lacadena *et al.* (1984). Statistical analysis of the distribution of nucleoli between different plants within each line was tested by applying χ^2-contingency tests.

RESULTS AND DISCUSSION

The results obtained for different combinations of tetraploid triticales are presented in table 1. Since no significant differences in the distribution of number of nucleoli between different plants for each combination analysed were detected, data were pooled. In tetraploids between *Aegilops squarrosa* and *Secale silvestre* DDR^sR^s and *Secale cereale* DDR^cR^c only one pair of chromosomes presented secondary constriction and positive Ag-NOR bands. The satellited chromosomes were identified with C-banding as belonging to *Aegilops squarrosa* (fig. 1). Since the maximum number of nucleoli observed was two, only the satellite chromosome pair from the D genome was active while SAT-chromosomes of rye remains inactive.

Nucleolus organising chromosomes from *A. squarrosa* are strong enough to suppress the nucleolar activity not only of NORs in cultivated rye but also those of the wild rye *Secale silvestre.*

In $(A/B)(A/B)R^cR^c$ tetraploid triticales with A/B mixed genomes, in which both satellited chromosome pairs from the B genome were present, four secondary constrictions were detected by phase contrast. The chromosomes exhibiting secondary constrictions were identified by C-banding as chromosome pairs 1B and 6B (fig. 2 a, b). Furthermore, the maximum number of nucleoli observed at interphase was four, which corresponds to the number of positive Ag-NOR bands detected, meaning that chromosome pairs 1B and 6B are responsible for organising nucleoli. Nucleolar activity of satellite chromosome pair 1R from rye is suppressed as well as NORs belonging to the A genome of wheat.

These results are in agreement with previous data reported for different wheat-rye-hybrids carrying 1, 2 or 3 doses of rye genome (Cermeño *et al.*, 1984; Lacadena *et al.*, 1984). However, in these materials at least one copy of each chromosome from the A and B genome of wheat was present while in the tetraploid triticales with A/B mixed genomes not all chromosomes of wheat were present.

The question arises, whether nucleolar compensation of minor NORs appears when major NOR chromosomes are lacking.

In combinations missing SAT-chromosome pair 6B, only 1B was active in producing nucleoli and showed secondary constrictions and strong Ag-NOR bands (fig. 2c, d).

These results agree with data obtained for different nullitetrasomic lines of wheat and aneuploid lines of hexaploid triticales lacking SAT-chromosomes 1B and 6B (Thomas and Kaltsikes, 1983). Thus, it must be concluded that in this case nucleolar compensation of minor NOR-chromosomes does not exist.

When SAT-chromosomes 1B and 6B are missing, chromosome pair 1R of rye was active to compensate major NOR deletions, exhibiting secondary constriction in nucleolar organiser regions and presenting strong Ag-NOR bands (fig. 2 e-g). Since the maximum number of nucleoli observed in this case was two, nucleolar activity of SAT-chromosomes from A genome of wheat remained suppressed.

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