Theor Appl Genet (1993) 87:17–21

THEORETICAL AND APPLIED GENETICS

Synaptic patterns of rye B chromosomes. II. The effect of the standard B chromosomes on the pairing of the A set

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Received: 4 February 1993 / Accepted: 1 March 1993

Abstract. In order to elucidate the possible effects of rye B chromosomes (Bs) on synapsis and metaphase-I associations of the A set, a comparative study between pachytene and metaphase-I-cells of rye plants carrying different numbers of Bs (0–8) has been carried out. The number of Bs was found to be positively correlated with the frequency of synaptic irregularities of the A set, i.e. multivalents and foldback pairing, and with the frequency of pachytene interlockings. It is proposed that interlockings are the origin of these irregularities because both appeared in close proximity in many nuclei. Examples of A-B pairing are described. The frequency of synaptic abnormalities seems to be unrelated to the mean of A-chromosome-bound arms at metaphase I.

Key words: Rye – B chromosomes – Synapsis – Interlocking – Non-homologous pairing

Introduction

In the extensive literature that is available on the B chromosomes (Bs) there are many reports on their effect on chromosome pairing of the A set. Where this type of study has been carried out in plants, the term "chromosome pairing" has led to confusion, since all of the data are derived from metaphase-I observations, and the conclusions that can be drawn are therefore related to effects on chiasma frequency or, more accurately, on chromosome associations at metaphase I, but never to effects on the synaptic process of the normal complement.

Rye Bs are among the better studied chromosomes, and information is available on their structure, effects and evolution. Studies on the influence of the Bs on chiasma frequency of the A set have produced contradictory results. Jones and Rees (1967) found that the Bs did not influence the mean chiasma frequency, but they did increase the variance in a zig-zag manner depending on whether the number of the Bs was odd or even, whereas Zecevic and Paunovic (1969) showed that the Bs increase the overall chiasma frequency per cell. Recently, the existence of a promoting effect on meiotic chromosome association located in the short arm of the Bs has been proposed (Alvarez et al. 1991). On the other hand, Tsumoto and Sasaki (1972) reported one rye line in which chiasma frequency decreased when the number of the Bs increased. It must be said, however, that all of these authors used different strains of rye, so their results are not directly comparable.

The existence or non-existence of a relationship between these effects and the primary pairing behaviour displayed by the A set at zygotene and pachytene remains unknown. In order to elucidate this relationship we have carried out a comparative study between pachytene and metaphase-I cells of rye plants from the Korean cultivar 'Puyo', which carries different numbers (0–8) of standard Bs.

Materials and methods

Plants of the Korean cultivar 'Puyo' carrying different numbers (0–8) of standard Bs were used. Single anthers of the emerging spikes were squashed in 2% acetic orcein to identify the pachytene stage of meiosis. The two sister anthers of the same floret were then prepared for synaptonemal complex (SC) isolation, as described by Holm (1986), with minor modifications. Surface-spread preparations were silver stained by the method of Loidl (1984).

Pollen mother cells of plants with different number of Bs were screened in electron micrographs. A total of 208 pachytene

Communicated by F. Mechelke
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nuclei were chosen for the present study. The criterion for pachytene was full synapsis of those bivalents or bivalent regions of the A set not involving in interlockings.

For metaphase-I observations, anthers at the proper stage were fixed in 1:3 acetic acid: ethanol and stored at 4 °C for several months until required. The fixed material was squashed and C-banded using the Giemsa staining procedure of Giráldez et al. (1979).

Results

Pachytene observations

Three hundred pachytene cells from 15 plants with different numbers of Bs [3 (0 Bs), 2 (1 B), 3 (2 Bs), 1 (3 B), 3 (4 Bs), 1 (5 B), 1 (6 B), 1 (8 B)] were monitored by electron microscopy. In the nuclei of 208 of these the SCs of both the standard set and the Bs, and the different types of synaptic abnormalities involving the A chromosomes (As), could be analysed.

Table 2. Mean frequencies of A-chromosome-bound arms at metaphase I in plants[a] with different numbers of standard B chromosomes

Number of Bs	Bound arms per cell	Number of cells
0	12.52±0.27	300
2	12.80±0.09	300
3	13.72±0.08	40
4	12.98±0.23	285
5	13.32±0.07	172
6	13.44±0.04	222
8	11.51±0.19	57

[a] Plants are the same as in Table 1

The most common abnormalities were multivalents and intrachromosomal non-homologous pairing (Figs. 1 and 5). The frequency of interlockings (ILs) was also quantified. Observations from different plants with the same number of Bs were pooled, since no striking differences among them were observed (Table 1). Some interesting findings are indicated by these results; namely, that there are positive correlations between: (1) the number of Bs and the mean of ILs ($r=0.95$); (2) the mean of ILs and the mean of A synaptic irregularities (multivalents and foldbacks) ($r=0.96$); (3) the number of Bs and the mean of synaptic irregularities ($r=0.94$); (3) the number of Bs and the mean of synaptic irregularities ($r=0.94$). That is, the Bs have a dosage effect on the number of ILs at pachytene and consequently, on the frequency of synaptic irregularities. In fact, ILs and synaptic abnormalities appear to be in close proximity in many nuclei (Figs. 2–4).

ILs are also the origin of short SC stretches between the Bs and any part of the A chromosome set that were occasionally observed (Fig. 4). This pairing is not a reflection of homology.

There was no suggestion that the Bs of rye have an effect on the synaptonemal complex length of the A chromosomes. The A set SC lengths were 534.44 μm±2.35,

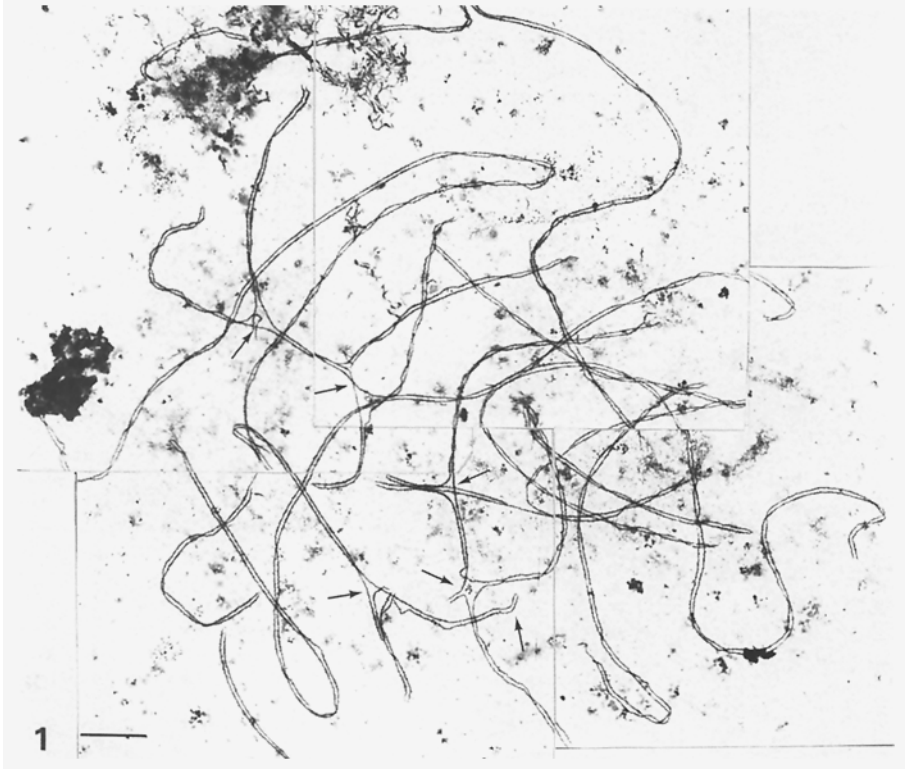

Fig. 1. Pachytene nucleus in a plant with four Bs in which there are many abnormalities. Some partner switches and stretches of non-homologous pairing are indicated by *arrows*. *Bar*: 10 μm

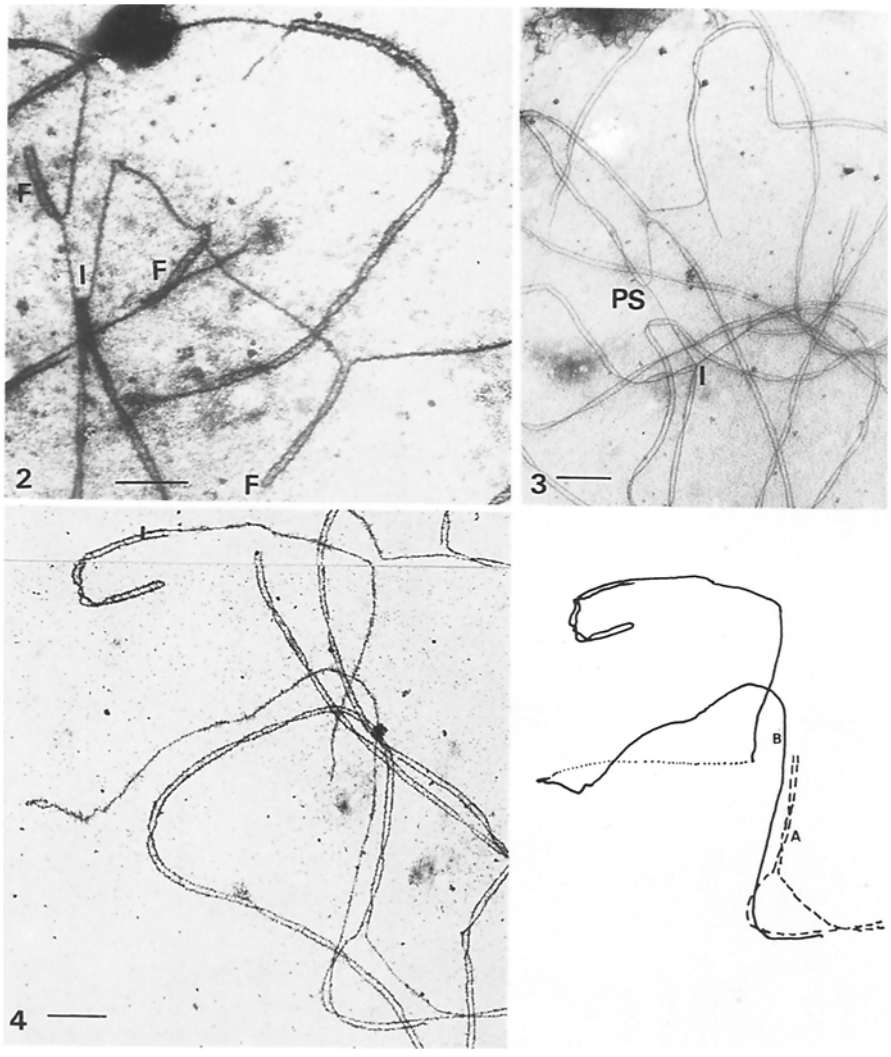

Fig. 2. Foldback pairings in an A bivalent associated with interlocking (*I*). *Bar*: 5 μm. **Fig. 3.** Partner switch (*PS*) associated with interlocking (*I*). *Bar*: 5 μm. **Fig. 4.** A-B synapsis associated with interlocking. *Bar*: 5 μm

Table 1. Mean frequencies of pachytene synaptic abnormalities involving A chromosomes in plants with different numbers of standard B chromosomes

Number of Bs	Fold-backs	Multi-valents	Total abnor-malities	Inter-lockings	Number of cells
0 (3)[a]	0.19	0.03	0.23	0.66	30
1 (2)	0.47	0.17	0.40	1.20	20
2 (3)	0.22	0.16	0.40	1.20	30
3 (1)	0.60	0.40	0.50	1.60	10
4 (3)	0.23	0.24	0.42	1.50	82
5 (1)	0.56	0.34	0.60	1.50	18
6 (1)	1.00	0.40	0.80	2.10	10
8 (1)	0.80	0.40	0.80	2.40	8

[a] The numbers of plants analyzed are in brackets

575.30 μm ± 2.79 and 571.69 μm ± 1.69 in plants carrying zero Bs, two Bs and four Bs, respectively ($F = 1.53$, non-significant at 5% level).

Metaphase-I observations

The mean frequencies of A-chromosome-bound arms at metaphase I in plants with different number of Bs are shown in Table 2. No correlation between parameters was detected ($r = 0.27$). If the plant carrying eight Bs is excluded from the analysis, there is a trend towards an increase in the mean of A-bound arms per cell when the number of Bs is higher, although the correlation coeficient is non-significant ($r = 0.69$).

No evidence of metaphase-I complex multivalents was found.

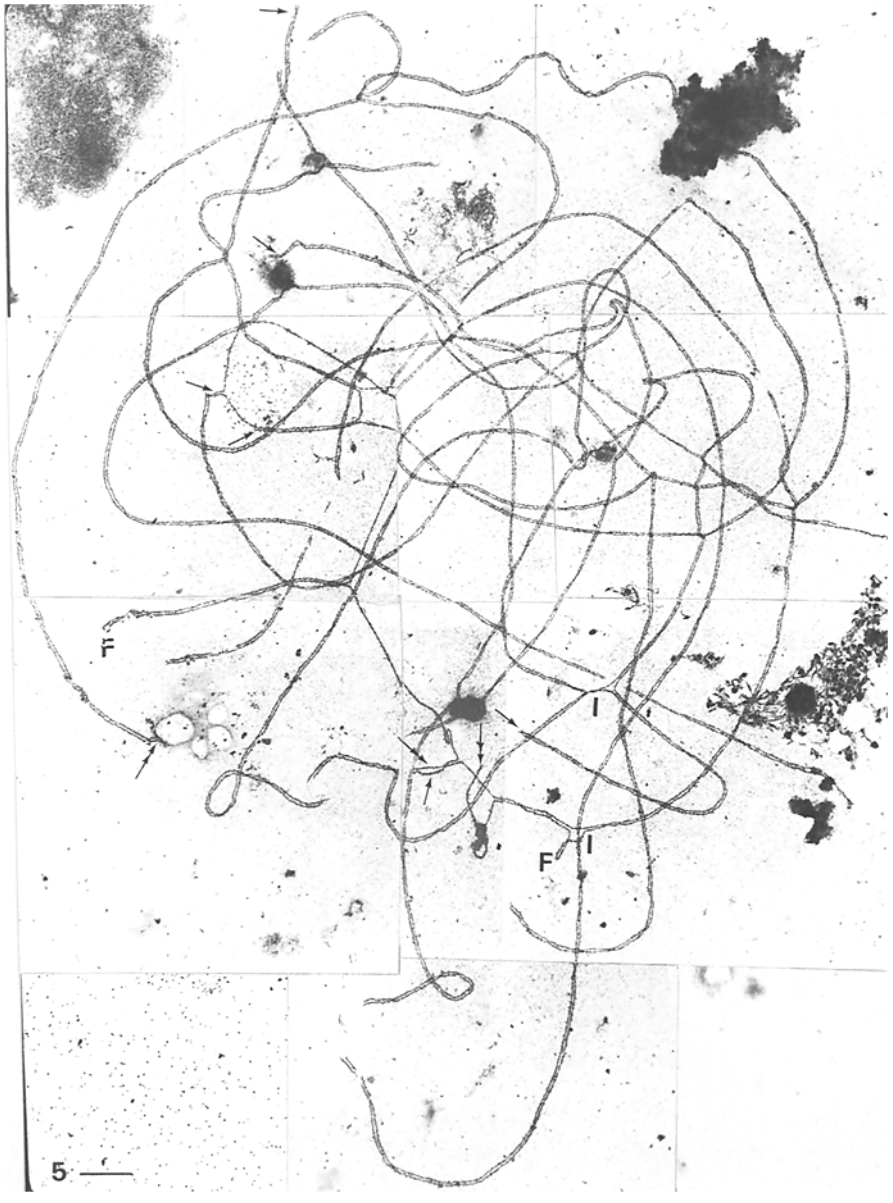

Fig. 5. Pachytene nucleus of a plant with 5 Bs in which seven chromosomes are associated (5 Bs+2 As). *Arrows* indicate the ends of the Bs, and *double arrows* indicate the ends of the A bivalent. Interlockings (*I*) and foldback pairings (*F*) are also visualized. *Bar*: 10 μm

Discussion

Crepis capillaris is the only species in which the effect of Bs on the pairing of the standard set has been studied. In this species Bs have a remarkable effect on increasing the SC length of the A chromosomes (cited in Jones et al. 1989). This contrasts with the present work where the synaptonemal complex of the A set from plants with two or four Bs is longer than that in plants with zero Bs; however, the high between-cell variances make these differences non-significant at the 5% level. Furthermore, Jones et al. (1991) also found that in plants with 4 Bs the pairing of the A set was frequently incomplete; nonhomologous foldback pairing of regions that had failed to pair homologously and abnormalities of SC structures such as the thickening and splitting of axes were also observed.

The effect of rye standard Bs on the pairing of the A set seems to be through the increase in the number of ILs that remain at pachytene. Chromosome interlockings (entrapments of chromosomes between unpaired interstitial segments of bivalents), and bivalent interlockings (entrapment of bivalents) are frequent at zygotene, and are presumably resolved before pachytene by the breakage of one or both lateral components and reunion of the broken ends (von Wettstein et al. 1984; Rasmussen, 1986; Santos et al. 1993).

Pachytene ILs bear the existence of unsynapsed regions around them that will try, at this stage, to satisfy pairing requirements independently of homology (von

Wettstein et al. 1984). This leads to multivalent and intrachromosomal foldback formation in which the Bs sometimes are involved (Figs. 4 and 5). Therefore, the examples of A-B pairing are of a non-homologous nature and do not reflect primary homology. This is the second case in which evidence of this type of pairing is presented; the first one was in maize where a B chromosome paired over a short distance with an extra heterochromatic region (k10L) of one of the standard chromosomes (Gillies 1983).

The effect of rye Bs on the pairing of the A set could be considered as a simple mechanic effect; that is, the effect is not produced by the intrinsic characteristic of these chromosomes but by their condition of having extra material that hinders the synaptic process. Thus, the higher the number of Bs the higher the number of ILs at zygotene, and the higher number of unresolved pachytene ILs implies a higher number of synaptic abnormalities.

On the other hand, the effect of Bs on the mean of A chromosome associations at metaphase I reported in other rye lines and cultivars is not evident in 'Puyo' and, if anything, it does not seem to be related to their influence on the pairing process of the A set. There was an absence of metaphase-I A multivalents, which were relatively frequent at the pachytene stage (Table 1). Restriction of chiasmata to homologously paired axial segments and/or multivalent SC corrections (Rasmussen and Holm 1979) could be responsible for these findings.

Acknowledgements. The authors wish to thank Dr. R. N. Jones for reviewing this paper and for his useful comments. G. Jiménez, A. Fernández, and the staff of the Complutense University Electron Microscope Unit, especially Dr. C. Barba, provided valuable assistance. This paper was supported by a grant PB 88-0121 awarded by the Comisión Interministerial de Ciencia y Tecnología (Spain).

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