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Short Communication

The nature and destiny of translocated B-chromosome-specific satellite DNA of rye

Robert Hasterok[1], Glyn Jenkins[2], Tim Langdon[2] & R. Neil Jones[2]
[1]*Department of Plant Anatomy and Cytology, University of Silesia, Jagiellonska 28, 40-032 Katowice, Poland; Tel: +48 (32) 2009 553; Fax: +48 (32) 255 58 73; E-mail: hasterok@us.edu.pl;* [2]*Institute of Biological Sciences, University of Wales, Aberystwyth, Ceredigion, SY23 3DD, UK; Tel: +44 (1970) 622 293; Fax: +44 (1970) 622 307*

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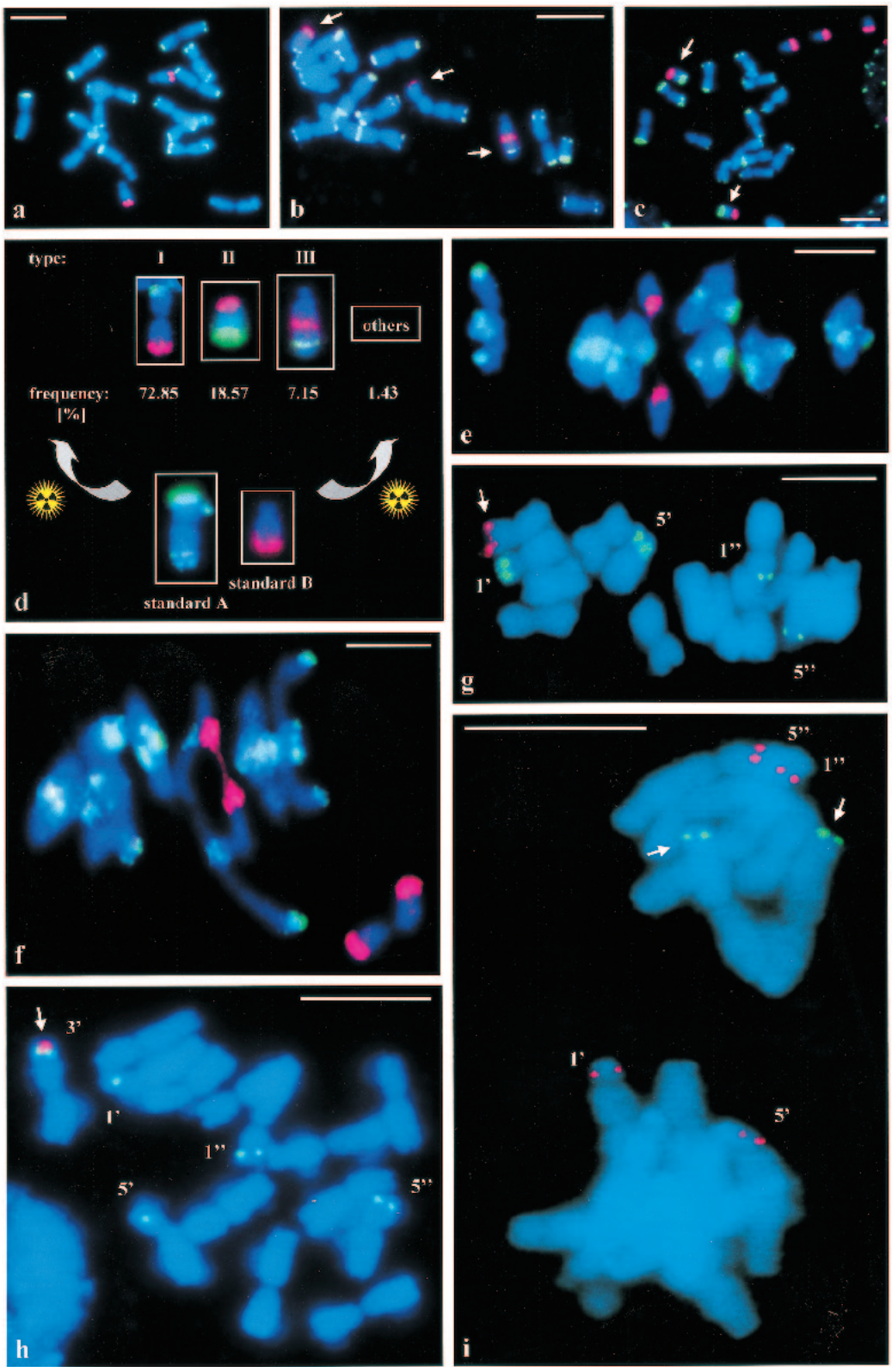
Abstract

Translocations of A chromosome-specific and B chromosome-specific satellite DNA were tracked by fluorescence *in situ* hybridisation from an irradiated M_1 generation of an experimental population of rye (*Secale cereale* L.) to its M_2 progeny. Although high frequencies of large structural rearrangements were detected in root-tip meristems of M_1 plants, none was present at meiosis or in somatic cells of their progeny. These results are interpreted in terms of efficient ''filtering'' of translocations during vegetative development, and not in the more usual terms of meiosis presenting a physical barrier to structural variants. These observations highlight the fact that B–A translocations are not tolerated, and may explain why this form of chromosome mutation is largely absent from natural populations.

B chromosomes (Bs) are dispensable and supernumerary to the basic A chromosome (A) set. They are present in many populations of rye (*Secale cereale*; $2n = 2x = 14 + Bs$) and, in contrast to the As, appear to lack genes, a characteristic which makes their function, if any, enigmatic. The overall DNA composition of As and Bs is similar (Tsujimoto & Niwa 1992), although each carries repeat families which are characteristic of the two chromosomal types. A B-chromosome-specific region occupying a block of heterochromatin at the distal end of the long arm (Wilkes *et al.* 1995, Langdon *et al.* 2000) is of particular significance since it is implicated in the control of the directed non-disjunction of chromatids at the first microspore division (Jones & Rees 1982).

This report uses fluorescent probes to A- and B-chromosome-specific repeats to track artificially induced B–A translocations from one generation to the next, the rationale being that we would be able to assay the cytological effects of restructuring the genome of rye. In particular, we were interested to know how additional pieces of B-chromosome-specific DNA would modulate recombination patterns and impart non-Mendelian behaviour to the As. Although a high


a
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frequency:
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72.85
18.57
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standard A
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frequency of translocations was induced between the two chromosome types, this approach was, to a certain extent, thwarted by the 'disappearance' of most structural variants before meiosis of the M_1 generation. However, these observations may have important implications in terms of intolerance of this form of chromosome mutation in natural populations.

Seeds of experimental B rye (*Secale cereale* L.; $2n = 2x = 14 + 0–4B$) were irradiated by 200 Gy of ^{60}Co gamma radiation. Root meristems of M_1 seedlings were prepared as substrates for fluorescence *in-situ* hybridization (FISH; Hasterok *et al.* 2001) and the individual plants grown to maturity. Immature inflorescences were also prepared for FISH, and individual plants selfed to create progeny homozygous for translocations in the M_2 generation. An A-chromosome-specific subtelomeric repeat sequence (pSc200; Vershinin *et al.* 1995), B-chromosome-specific subtelomeric repeat sequence (D1100; Sandery *et al.* 1990) and 5S rDNA (pTa794; Gerlach & Dyer 1980) were amplified in the presence of either rhodamine-4-dUTP (Amersham) or digoxigenin-11-dUTP (Roche) from plasmids, using PCR with universal M13 primers under standard conditions. FISH was performed according to Hasterok *et al.* (2001).

Figure 1a shows a typical C-metaphase spread of chromosomes from an individual of a control (non-irradiated) population, which has been probed with the A- (green) and B- (red) chromosome-specific repeat sequences. The former is confined to most of the subtelomeric heterochromatic regions of the A chromosomes only, and is entirely consistent with published data (Vershinin *et al.* 1995). The latter occupies the distal regions of the B chromosomes only and is also consistent with previous observations (Wilkes *et al.* 1995, Langdon *et al.* 2000).

The root meristems of a total of 13 M_1 seedlings were screened by FISH for B–A translocations. Eight (61.5%) plants had detectable rearrangements, from which six were selected on the basis of their relatively high proportions of translocated C-metaphases, ranging from 10 to 78% of the sample of C-metaphases from individual plants. Figures 1b and 1c show the commonest types of translocation detected in a sample of 174 C-metaphases from the six plants. Figure 1d shows the relative frequencies of these, and their classification into distinct types. In type I, a single prominent block of B-chromosome-specific DNA is translocated to one arm of an A chromosome. Since the shape of the chromosome appears unchanged, the assumption is that the translocations in these cases are reciprocal. Type II translocations create chromosomes with indistinct primary constrictions and substantial blocks of D1100 and pSc200 repeats at opposite ends. Type III appears to be largely an intact B chromosome to which a large block of A chromatin has been translocated to the distal part of its long arm. Other structural variants are relatively rare and constitute less than 2% of those observed.

Metaphase I of meiosis was studied in all six plants known to have high frequencies of translocations in their root-tip meristems. Surprisingly, no rearrangements were detected at this stage. Meiosis is apparently normal, with A and B chromosomes forming separate bivalents (Figure 1e, f) or the latter failing to pair and forming univalents (Figure 1f).

Cytological screening of root-tip cells of 64 individuals of the M_2 generation derived from selfing M_1 plants known to harbour chromosomal rearrangements in somatic tissue revealed no large B–A translocations. However, in all C-metaphases of 22 (34.4%) plants, small fragments of B-specific chromatin were detected at the distal tip of either one (Figure 1g, h) or two (Figure 1i) A chromosomes of the complement. In some cases, these D1100 sites can be assigned to particular chromosomes by colocalisation with other chromosomal

Figure 1. FISH of A- (green) and B- (red) chromosome-specific repeats to C-metaphase chromosomes of a control (non-irradiated) plant (**a**) and M_1 generation (**b,c**). The classification and relative frequencies of the three most numerous types of translocation are shown in (**d**). (**e–f**) FISH of D1100 B-specific (red) and pSc200 A-specific (green) sequences to metaphase I of meiosis in pollen mother cells of the M_1 generation. Note the B rod bivalent with a distal chiasma in the long arm and the two B univalents in (**f**). (**g–i**) FISH of the B-specific repeat (red) and 5S rDNA (green) to C-metaphase chromosomes of the M_2 generation. In (**i**), the colours are reversed. The identities of the chromosomes with 5S rDNA loci are indicated. Note the colocalization of translocated B-chromosome-specific DNA and rDNA on chromosome 3 in (**h**). B–A translocations are indicated by arrows. Bar equals 10 μm.

landmarks, such as 5S rDNA (Figure 1h) which is borne on chromosomes 1R, 3R and 5R (Cuadrado & Jouve 1997).

Spontaneous reciprocal or non-reciprocal B–A rearrangements occur seldom except under experimental conditions (Puertas & Baeza 1983). It has always been surmised that such rearrangements would compromise meiosis, and that the natural filter which meiosis provides for the proper function of its processes eliminates such mutations as quickly as recurrent chromosome mutation generates them. However, our study indicates that it is not meiosis but more probably the vegetative phase of plant development which 'filters out' structural chromosome variants. It appears that only relatively small fragments of B-specific chromatin at subtelomeric regions of As might be able to pass through meiosis without pairing disruption and be stabilized in the following generations. A less likely but plausible explanation is that the chromosomes of root primordia of embryos are more vulnerable to damage by radiation than cell lines destined for aerial and reproductive parts of the plant. This could effectively segregate structural variants of chromosomes to particular plant tissues. Similar karyotypical studies of the root meristems of mature M_1 plants may help to distinguish between these two possibilities. Nature's stringency on the permissible limits of chromosome mutation, in relation to that between A and B chromosomes, has been confirmed by experimentation. The A and B chromosomes of rye are adapted to separate tracks of inheritance, and it seems that there can be no crossing of the genome barrier.

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