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Sister Chromatid Exchanges in Barley

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Summary. A mean frequency of 20.6 sister chromatid exchanges (SCEs) per cell has been observed in a reconstructed karyotype of *Hordeum vulgare* by application of the FPG technique after unifilar incorporation of BrdU into chromosomes. The involvement in SCEs of the 48 segments into which the chromosome set had been subdivided was, with a single deviation, length proportional and independent of the segment's heterochromatin content. Asymmetric bands, indicative of an uneven distribution of adenine and thymidine between the DNA strands in adenine (A)-thymidine (T) rich chromosome regions, could not be detected after incubation of the cells in BrdU for one cycle of DNA replication.

Key words: SCE – Intrachromosomal distribution of SCEs – Barley

Introduction

Sister chromatid exchanges (SCEs) in plant chromosomes were first demonstrated by Kihlman and Kronborg (1975) on the basis of differential BrdU (bromodeoxyuridine) content in sister chromatids made visible by the FPG (fluorescence plus Giemsa)-technique (Perry and Wolff 1974). Since that time results obtained by the same method have been published for only three plant species, *Vicia faba* (Kihlman and Kronborg 1975; Kihlman 1975; Scheid 1976, Vosa 1976a; Kihlman et al. 1977; Kihlman and Sturelid 1978; Kihlman et al. 1978; Schubert et al. 1979 a, b), *Allium cepa* (Schvartzman and Cortes 1977) and *Secale cereale* (Friebe 1978).

Here we wish to report on the frequency and intrachromosomal distribution pattern of SCEs in barley chromosomes unifilarly substituted with BrdU. The barley karyotype used was structurally reconstructed and characterized by 7 interdistinguishable pairs of chromosomes due to the presence of two homozygous translocations (Nicoloff et al. 1975; Künzel and Nicoloff 1979).

Additionally, we looked for asymmetric bands which may appear when cells are fixed after one round of DNA replication (12 h) in BrdU. These asymmetric bands are presently known for man, mouse and *Vicia faba* chromosomes (for review see Schubert and Rieger 1979) and are interpreted to be indicative of AT-rich chromosome regions with an uneven distribution of A and T bases between the polynucleotide strands of the DNA double helix.

Material and Methods

The Karyotype

The barley karyotype used (MK 14/26) is characterized by the presence of two homozygous translocations, one involving chromosomes 2 and 7, the other chromosomes 3 and 4 (Fig. 1, Nicoloff et al. 1975; Künzel and Nicoloff 1979)

Treatment Procedures

Roots of germinated seeds were initially treated (at 24° in darkness) with 5-bromodeoxyuridine ($5 \cdot 10^{-4}$M), fluorodeoxyuridine ($5 \cdot 10^{-8}$M) and uridine (10^{-6}M) for 12 h and then with thymidine ($2 \cdot 5 \cdot 10^{-5}$M) and uridine (10^{-6}M) for another 12 h. They were then immersed in colchicine (0.05%) for 3 h and subsequently fixed in ethanol: acetic acid (3:1). The making of squashes and remainder of the FPG-technique was done as described for *Vicia faba* by Schubert et al. (1979 a). For detection of asymmetric bands the incubation in BrdU was immediately followed by colchicine treatment. The remaining procedures were the same as those used for SCE detection.

Statistics

Each of the centromeres and nucleolus organizing regious (NOR), whose sizes are difficult to measure exactly, was estimated to contain 1% of the metaphase genome length. The rest of the ge-

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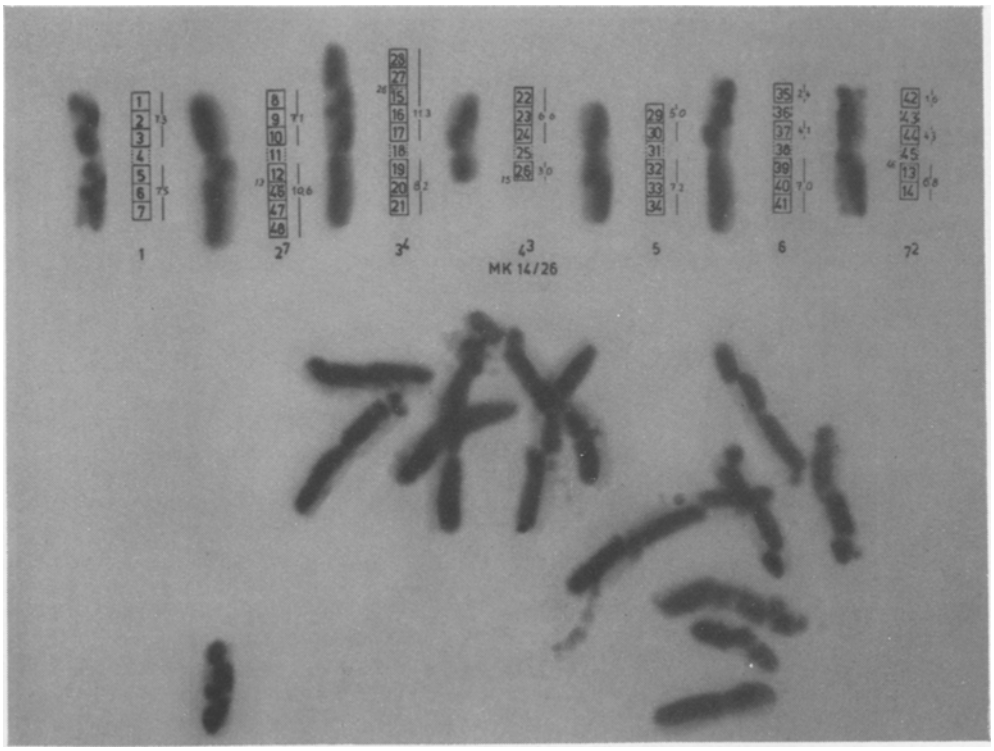

Fig. 1. Barley karyotype MK 14/26; individual chromosomes with SCEs, the mode of segment subdivision and the relative length of arms. The segment sequence is indicative of the points of translocations in chromosomes 3 and 4 as well as 2 and 7. The combination of these two translocations resulted in the reconstructed karyotype MK 14/26. Broken lines symbolize centromeres (segments 4, 11, 18, 25, 31, 38 and 45) and nucleolar organizers (segments 36 and 43), respectively (upper part of Fig. 1). A complete metaphase with chromosomes showing SCEs (lower part of Fig. 1)

nome was proportionally attributed to the other 39 segments (for relative arm length and segment sequence see Fig. 1).

Since the number of SCEs is assumed to be a random variable with a binomial distribution, upper and lower confidence limits for length proportional distribution of SCEs were calculated for each segment at a significance level of 1% (for details see Rieger et al. 1975).

Results and Discussion

SCE Frequency

After unifilar BrdU incorporation a mean frequency of 20.6 SCEs per cell was observed. Similar treatment resulted in 20-30 SCEs per cell in *Vicia faba* (Kihlman and Kronborg 1975; Schubert et al. 1979 a), and in 44.8 SCEs in *Allium cepa* (Schvarzman and Cortes 1977). Incubation in BrdU for two cycles of DNA replication produced 11.06 SCEs per cell in *Secale cereale* (Friebe 1978). Since it proved difficult to find enough complete metaphase cells with sister chromatid differentiation in barley, we calculated the SCE frequency per cell by evaluating equal numbers (100) of individual chromosomes.

Inter- and Intrachromosomal Distribution of SCEs

The 48 individual chromosome segments, into which the barley karyotype was subdivided, showed, with one exception, that length was proportional to involvement in SCEs (Fig. 2, 1% significance level). This inference is based on the localization of 1030 SCEs in a quantity of chromosomes which corresponded to 50 complete metaphases from 10 different roots. The only deviation from this pattern occurred with respect to segment 35 (the satellite of chromosome 6) which was found to be less frequently involved in SCEs than expected on a length-proportional basis. At present we have no reasonable explanation for this deviation. In segment 35 no noticeable amount of heterochromatin was found by means of the Giemsa-technique (Vosa 1976b; Linde-Laursen 1978), whereas the segments containing heterochromatin (in barley, mainly pericentric, but in some cases also telomeric and intercalary chromosome regions), which are underrepresented with respect to SCE involvement in *Allium* (Schvartzman and Cortes 1977) and *Secale* (Friebe 1978), showed no significant deviation from length-proportional SCE distribution in barley. The nucleolus or-

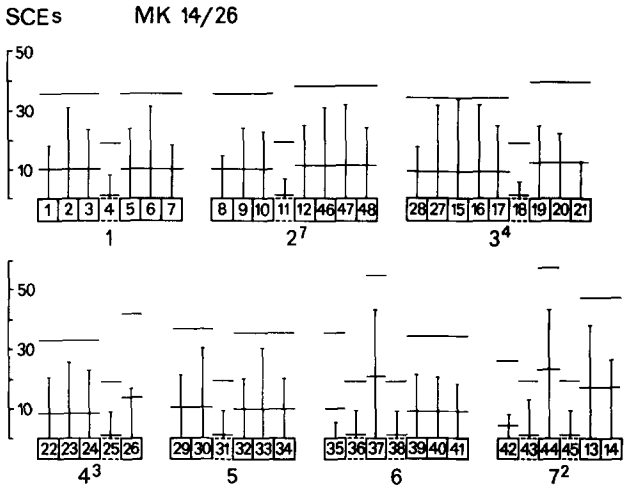

Fig. 2. The absolute involvement in SCEs and the upper and lower confidence limits for length proportional SCE distribution (1% significance level) for each of the 48 segments of karyotype MK 14/26. Broken lines symbolize centromeres and nucleolar organizers, respectively

ganizing secondary constrictions of barley, regions which in *Vicia faba* represent SCE hot spots (Schweizer 1973; Schubert et al. 1979a), also did not surpass the confidence limits for length proportional involvement in SCEs.

Asymmetric Bands

Unifilar incorporation of BrdU into both chromatids resulted in asymmetric dark bands in weakly stained chromosomes of *Vicia faba* (Schubert and Rieger 1979); no corresponding bands were observed in barley. The reason for this might be either the absence of AT-rich sequences with uneven distribution of adenine and thymidine between the two polynucleotide strands of the DNA double helix in satellite-DNA of barley (about 4% of the genome; c.f. Peacock et al. 1978) or, if existent, such sequences might be so short and widely dispersed that the power of resolution of the method used is insufficient to detect them.

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