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Effects of Chromosome Reconstruction on Deletion Clustering in *Hordeum vulgare*

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Summary. The barley standard karyotype, two reconstructed karyotypes with all chromosomes interdistinguishable, and four translocation lines were treated with maleic hydrazide. A specific chromosomal site in satellite chromosome 7 (segment 44 adjacent to the nucleolus organizer region) of the standard karyotype was found to represent a deletion hot spot. A sample of specifically reconstructed karyotypes were used to check whether or not transposition of the hot spot region, or changes of its neighborhood, would affect its involvement in deletions. One of the seven karyotypes (translocation line T 505 with a pair of chromosomes having both nucleolus organizer regions and satellites in opposite arms) was without deletion clustering in segment 44. At the same time, a prominent Giemsa band close to the secondary constriction was absent from segment 44. These data show that the involvement in deletions of a certain chromosome segment is modifiable in certain cases by chromosome reconstruction. Similar observations have been made in *Vicia faba*.

Key Words: Chromatid aberrations – Deletion clustering – Maleic hydrazide – Translocation karyotypes – Giemsa banding – *Hordeum vulgare*

Introduction

We previously reported on clustering in specific chromosome segments of intercalary deletions in barley, and of duplication-deletions as well as intercalary deletions in *Vicia faba* (Nicoloff et al. 1979). This preferential involvement in specific types of chromatid structural changes was mutagen-dependent in barley but mutagen-independent in *Vicia faba*. After treatment of barley with maleic hydrazide and mitomycin C, deletion clustering occurred in a specific region (segment 44) of chromosome 7 (one of the satellite chromosomes); no preferential involvement in deletions of this or any other chromosome region was observed when ethylene imine or trenimone were used to induce chromatid structural changes.

When the chromosomal position of the segment preferentially involved in deletions in *Vicia faba* was changed by karyotype reconstruction via reciprocal translocations, its involvement was found to be quantitatively modifiable in certain cases of positional change: there was some sort of 'position effect' (Rieger et al. 1977). Some translocation lines were used to look for similar effects on deletion clustering in barley. In this paper we will show that deletion clustering can also be affected in barley by appropriate chromosome reconstruction.

Material and Methods

The barley standard karyotype, two reconstructed karyotypes and four single translocation lines, were used for intrachromosomal localization of chromatid aberrations induced by maleic hydrazide. The reconstructed karyotype MK 14/26 of barley has previously been described (Nicoloff et al. 1975; Nicoloff and Künzel 1976). It originated from the combination of two reciprocal translocations involving chromosomes 2 and 7 and chromosomes 3 and 4 of the standard karyotype. Recently, another reconstructed karyotype of barley (MK 14/2034) has been produced which represents a further improvement with respect to the cytological identification of individual barley chromosomes (Künzel and Nicoloff 1979). Karyotype MK 14/2034 is characterized by the presence of reciprocal translocations involving chromosomes 1 and 7 and chromosomes 3 and 4. All chromosomes of these karyotypes are morphologically distinguishable.

The translocation lines used each involve a single reciprocal translocation between satellite chromosomes and non-satellite chromosomes of barley: segment 44 (the deletion hot spot in the short arm of chromosome 7) became translocated to new positions. In translocation line T 56, segment 44, together with the nucleolus organizer region (NOR) and the satellite, is translocated to the long arm of the non-satellite chromosome 5 (chromosome 5^7); in trans-

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location line T 506, segment 44, with the NOR and satellite of chromosome 7, is interchanged with part of the long arm of the satellite chromosome 6 (chromosome 6^7); in reconstructed karyotype MK 14/26, part of the long arm of satellite chromosome 7 is interchanged with part of the long arm of the non-satellite chromosome 2 (chromosome 7^2); while in translocation line T 505, part of the long arm in chromosome 7 is interchanged with segment 37 (short arm of chromosome 6), the NOR and the satellite (chromosome 7^6).

In reconstructed karyotype MK 14/2034, part of the short arm of chromosome 1 is translocated to the satellite of chromosome 7 (chromosome 7^1), and in translocation line T 21 a portion of the NOR of satellite chromosome 7 is interchanged with part of the short arm of chromosome 5 (chromosome 7^5 and 5^7, respectively).

Presoaked seeds (1 h in tap water) of the standard karyotype (var. 'Frigga'), the reconstructed karyotypes (MK 14/26 and MK 14/2034) and the four single translocation lines (T 56, T 506, T 21, T 505) were kept on moist filter paper for 18 h in an aerated desiccator. The seeds were subsequently treated with maleic hydrazide (2 h, 5×10^{-3} M at 24°C). After treatment, the seeds were germinated in petri dishes (24°C) and fixed (1:3 glacial acetic acid and ethanol) at different recovery times (15, 18, 21, 24, 27 h). Prior to fixation the roots were immersed (2 h) in a solution of 0,025% colchicine saturated with α-bromonaphtalene. After maceration of the roots in 45% pectinase, permanent Feulgen squashes were prepared by the dry-ice method of Conger and Fairchild (1953).

For the localization of aberrations the barley karyotypes were subdivided into 48 segments (Nicoloff et al. 1975; Nicoloff and Künzel 1976; Künzel and Nicoloff 1979) whose individual involvement in aberrations was tested. Four types of induced chromatid aberrations were scored and located per segment: isochromatid breaks (i), duplication-deletions (dd), intercalary deletions (d), and chromatid translocations (t) (Nicoloff and Gecheff 1968).

For Giemsa-banding of barley chromosomes the schedule described by Noda and Kasha (1978) was used with some modifications. Root tips, 6-8 mm in length, were pretreated for 2 h at 24°C with a solution of 0.025% colchicine saturated with α-bromonaphtalene, fixed in ethanol-glacial acetic acid (3:1) for 24-36 h and stored in 70% ethanol at 4°C. After rinsing in distilled water, the roots were placed in 1% pectinase for about 45 min. at 37°C; after squashing in 45% acetic acid the cover glasses were removed on dry ice and the slides air-dried for 24 h, hydrolyzed for 5 min. (1 n HCl at 60°C), rinsed in distilled water and air-dried at room temperature for 6 h.

Subsequently the slides were immersed in 0.07 n NaOH for about 20-30 sec and stained in 4% diluted Giemsa-solution in Sörensen-buffer (pH 6.7). After staining, the slides were rinsed in distilled water (pH 7.0), air-dried and mounted in Eukitt (Kindler, Freiburg i.Br.).

Results and Conclusions

The standard position of segment 44 is shown in Figure 1. It is a chromosome region adjacent to the centromere of chromosome 7. The nucleolus organizing region (segment 43) and the satellite of chromosome 7 are distally located. When the standard karyotype was treated with maleic hydrazide, segment 44 showed up with deletion clustering: 15.6% of all chromatid aberrations observed in this karyotype represent intercalary deletions involving segment 44 (80 deletions localized, 76 of these in segment 44).

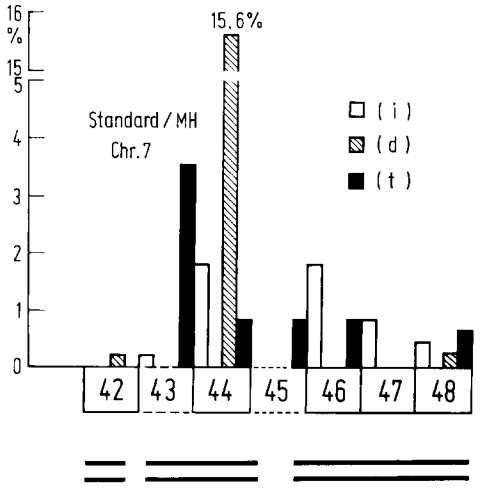

Fig. 1. The percent involvement in chromatid aberrations (isochromatid breaks (i); duplication deletions (dd); deletions (d); lesions involved in chromatid translocations (t)) of chromosome 7 (standard karyotype of barley), subdivided into 7 segments, after treatment with maleic hydrazide. The % of the Y axis is derived from the total number of aberrations scored for all 48 segments of the 7 chromosome pairs. Segment 44 is a deletion 'hot spot' (the deletions localized in this segment comprise 15.6% of all the aberrations scored). Arrows = translocation points

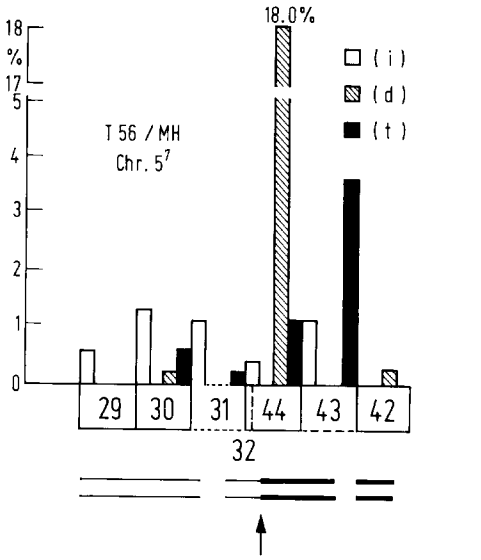

Fig. 2. Deletion clustering in segment 44 after treatment with maleic hydrazide of translocation line T 56. Due to interchange the segment sequence 42, 43, 44 is part of chromosome 5

The other karyotypes treated with maleic hydrazide allowed the examination of whether positional changes with respect to segment 44 might affect its involvement in deletion clustering. The results obtained are as follows:

1. If segments 42, 43, 44 are, due to reciprocal interchange, part of chromosome 5 (T 56, chromosome 5^7) segment 44 remains a deletion hot spot (Fig. 2).

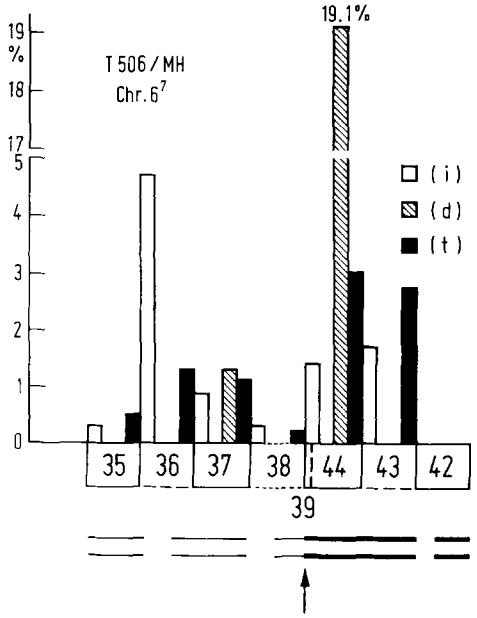

Fig. 3. Deletion clustering in segment 44 after treatment with maleic hydrazide of translocation line T 506. The interchange resulted in a chromosome 6^7 with both NORs and satellites of barley in opposite arms

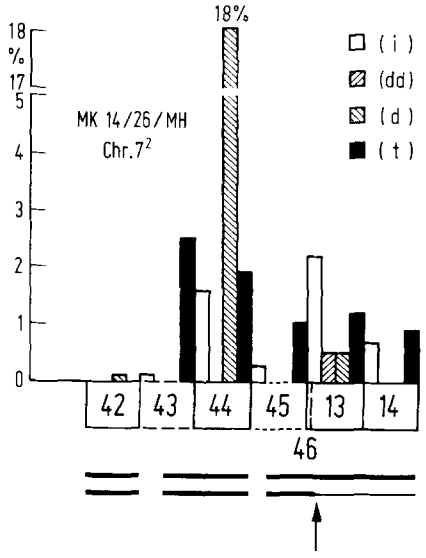

Fig. 4. The chromosome location of segment 44 in the reconstructed karyotype MK 14/26 (part of the long arm of the standard chromosome 7 has been interchanged for part of the long arm of chromosome 2). After treatment with maleic hydrazide segment 44 responds with deletion clustering

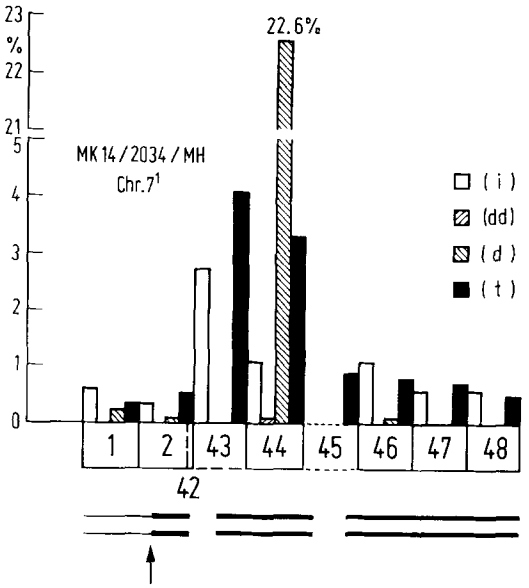

Fig. 5. Deletion clustering in segment 44 remains unaffected by translocation of part of the short arm of standard chromosome 1 to chromosome 7 after treatment with maleic hydrazide of karyotype MK 14/2034

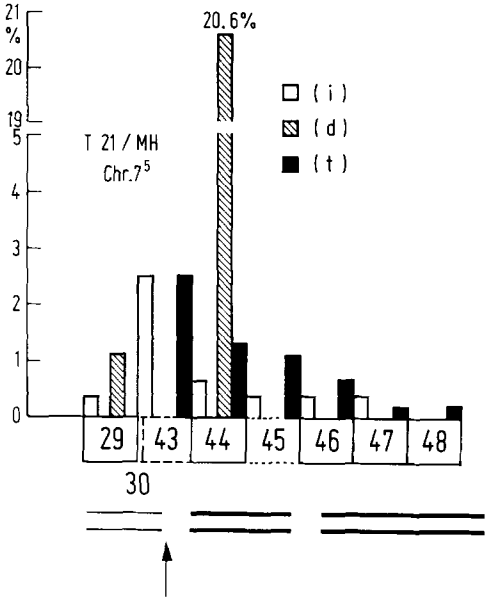

Fig. 6. Deletion clustering in segment 44 after treatment of translocation line T 21 with maleic hydrazide. In this line a portion of the NOR of standard chromosome 7 is interchanged with part of the short arm of chromosome 5

2. The same is true when segment sequence 42, 43, 44 is translocated to chromosome 6 (T 506; Fig. 3). This interchange results in a chromosome 6^7 which contains both NORs of the barley karyotype.

3. In karyotype MK 14/26 chromosome 7 interchanged a segment sequence of its long (non-nucleolar) arm for part of chromosome 2. The normal structure of the SAT-arm of chromosome 7^2 is upheld and the structural change once more is without influence on deletion clustering in segment 44 (Fig. 4). The same is true when a piece of chromosome 1 becomes translocated by reciprocal interchange to the satellite of chromosome 7 (karyotype MK 14/2034,

chromosome 7^1, Fig. 5) or when one of the break points of the reciprocal interchange is within the NOR, as examplified by translocation line T 21 in which a portion of the NOR and the satellite of chromosome 7 was interchanged for a piece of chromosome 5 (chromosome 7^5, Fig. 6).

4. Deletion clustering in segment 44 was, however, completely absent in translocation line T 505 (Fig. 7) in which segment sequence 35, 36, 37 with the NOR and satellite of chromosome 6 was interchanged for segment sequence 46, 47, 48 of chromosome 7. This interchange, similar to translocation line T 506 (Fig. 3), once more resulted in a pair of chromosomes with both NORs and satellites in opposite arms (chromosome 7^6). But while T 506 (chromosome 6^7) remained without influence on deletion clustering in segment 44, this time the same segment in chromosome 7^6 was no longer a deletion hot spot.

We previously studied the same two translocation lines with respect to nucleolus formation by chromosomes 6^7 and 7^6 and observed that two of the four NORs combined in a single pair of homologous chromosomes (either 6^7 or 7^6) become more or less completely 'repressed' and only produce micronucleoli (Nicoloff et al. 1977, 1979). Contrary to the situation with deletion clustering, both translocation lines were found to show a similar pattern of repression of NORs with the NOR of chromosome 6 exerting 'nucleolar dominance' over NOR 7 (Anastassova-Kristeva et al. 1980). Thus, the capability of nucleolus formation is evidently without influence on the differences observed with respect to delection clustering in these two translocation lines.

From Giemsa banding studies in barley (Linde-Laursen 1975, 1978a, b, 1979; Vosa 1976; Noda and Kasha 1978) it is well known that, dependent on the procedure used, Giemsa bands occur close to the secondary constriction of chromosome 6 (segment 37) and 7 (segment 44). We looked for these bands in translocation chromosomes 6^7 (deletion clustering in segment 44) and 7^6 (no such clustering in segment 44). Both of these bands occurred in chromosome 6^7 (T 506), one was absent in 7^6 (T 505). The band lacking is the band close to the secondary constriction in segment 43 (Fig. 8). Concomitantly segment 44 no longer shows up with deletion clustering.

Causes underlying the disappearance of the Giemsa band in segment 44 are presently unknown. The band's disappearance and the fact that segment 44 is no longer a deletion hot spot in translocation line T 505 may, however, be taken to presume a causal relationship between the two phenomena. Further studies are necessary to either prove or disprove this speculation. If true such a causal relationship will provide new insights into the phenomenon of type-specific clustering of certain types of induced chromatid structural changes and their connections with heterochromatin made recognizable by application of the Giemsa banding technique.

In this connection it should be mentioned that the disappearance of the Giemsa band in segment 44 in translocation line T 505 may be due to some sort of 'position effect' coupled with the specific constitution of chromosome 7^6. This gains support from the heterozygous translocation line HT 505 containing the standard chromosomes

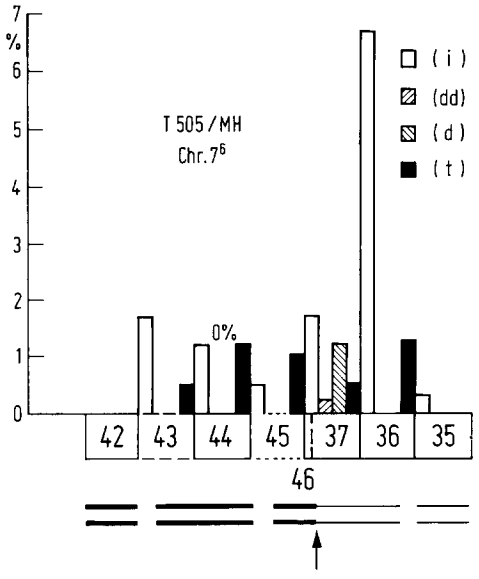

Fig. 7. Absence of deletion clustering in segment 44 after treatment with maleic hydrazide of translocation line T 505. In this karyotype part of the long arm of chromosome 7 is interchanged with segments 37, 36, and 35 of standard chromosome 6. The result is a chromosome 7^6 with both barley NORs and satellites in opposite arms

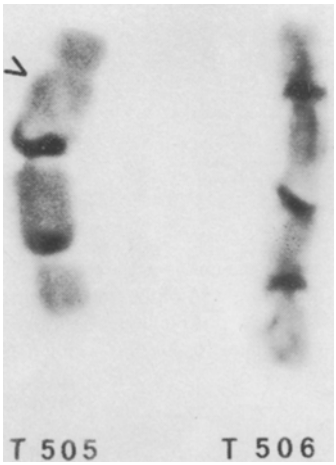

Fig. 8. Giemsa banding of chromosome 7^6 (T 505) and 6^7 (T 506) showing absence of one band (arrow) in segment 44 close to the secondary constriction in segment 43 of chromosome 7^6 (compare Figs. 3, 7). Concomitant with the absence of this band, segment 44 is no longer a deletion hot spot (Fig. 7)

6 and 7 (both satellite chromosomes) and the translocation chromosomes 6^7 (no NORs) and 7^6 (two NORs in opposite arms and no deletion clustering). In this line, chromosomes 6 and 7 showed a prominent Giemsa band adjacent to the secondary constriction; the Giemsa band in segment 44 of chromosome 7^6 was lacking in this karyotype.

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