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Further evidence for a high position-specific effect in the action of chemical mutagens on the chromosomes of barley

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Summary. Further convincing evidence for the decisive role of chromosome constitution in the processes underlying the specific induction of structural mutations by chemical mutagens is described. The most important conclusion to be drawn from the results obtained in experiments with maleic hydrazide (MH) and ethyl methanesulphonate (EMS) is that segments 39 and 47, situated next to the secondary constrictions of standard chromosomes 6 and 7, when tandemly combined by reciprocal translocation in chromosome 7^6 of reconstructed karyotype T-21, behave in a rather similar way. This is independent of the nature of the chemical mutagens applied, as far as the distribution pattern of induced chromatid aberrations is concerned. The phenomenon may be characterized as follows: (1) the segments in question in the new position appeared to be the most pronounced aberration "hot spots", the expressivity in segment 39 always being higher; (2) the pronounced "hot spot" character of these segments proved to be due mainly to their preferential involvement in intercalary deletions and duplication-deletions; (3) the specific constitution of chromosome 7^6 resulted in the majority of cases in a marked increase of the region involved in the aberration types mentioned above in segment 39. This is one of the very few examples of true position effect in the expression of chemically induced structural mutations at the chromosome level.

Key words: Chromosome aberrations – Translocation karyotypes – Maleic hydrazide – Ethyl methanesulphonate – *Hordeum vulgare*

Introduction

Mutagenic specificity may occur at different levels of the structural organization of genetic material (Auerbach and Westergaard 1960). The main evidence for the specific action of mutagens in both plant and animal cells has been established at the chromosomal level. In fact, the specific activity of chemical mutagens in particular chromosome regions proved to be one of their most characteristic features (cf. Kihlman 1966; Rieger and Michaelis 1967).

Our recent investigations in barley (Gecheff 1989) revealed a new manifestation of the mutagenic specificity. Using two reconstructed karyotypes that differ from one another in the position of the most pronounced "hot spot" segments, it was observed that the size of the chromosome region involved in mitomycin-C-induced intercalary deletions and duplication-deletions is significantly increased when the segments in question are situated tandemly in the reconstructed chromosome.

In this paper, the action of MH and EMS on the chromosomes of the same karyotype variants (T-1586 and T-21) is described. It was supposed that the application of mutagens with a different mode of action in this case may shed a bit more light on the possible nature of this phenomenon, which was thought to be caused by the specific alteration of the chromosome constitution.

Materials and methods

All data concerning the description of the plant materials (production of the translocation lines and position of translocation break-points), cytological techniques, scoring procedures, and statistical treatments used were given in a previous paper (Gecheff 1989).

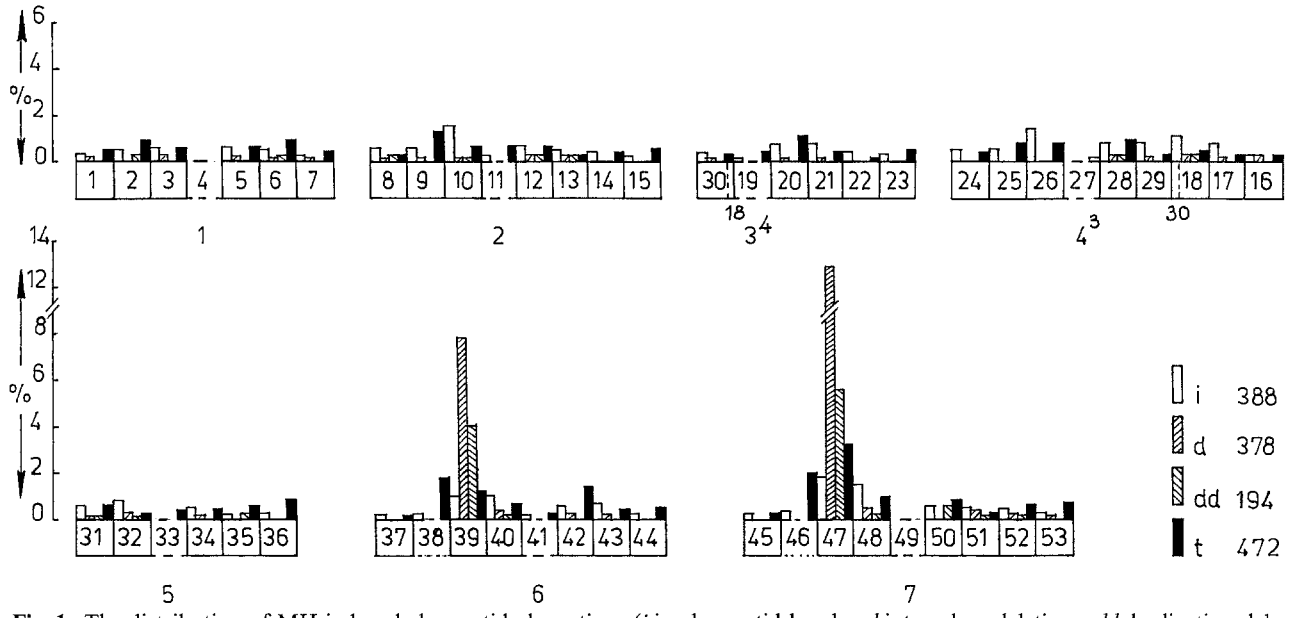

Fig. 1. The distribution of MH-induced chromatid aberrations (*i* isochromatid breaks, *d* intercalary deletions, *dd* duplication-deletions, *t* chromatid translocations) along the chromosomes of karyotype T-1586

Table 1. Frequency of chromatid aberrations induced by MH and EMS in barley karyotypes T-1586 and T-21

Treatment	Karyotype	Metaphase with aberrations at different recovery times (h) after treatment with mutagens (%)				
		15	18	21	24	27
MH	T-1586	27.3±4.5	38.5±4.3	43.3±4.5	39.8±2.8	25.8±3.3
	T-21	32.5±1.7	41.5±4.4	48.5±4.3	42.3±3.9	28.0±1.7
EMS	T-1586	26.4±2.0	30.3±3.5	38.1±2.7	36.2±3.0	28.0±2.6
	T-21	28.0±1.8	31.6±2.2	38.0±2.4	38.1±2.7	28.3±2.1

For mutagenic treatment, germinating seeds of karyotypes T-1586 and T-21 were immersed in aqueous solutions of MH (5×10^{-3} *M*, 2 h) or EMS (1%, 2.5 h).

All experiments were carried out in at least three replications.

Results

Frequency and intrachromosomal distribution of aberrations induced by MH

As can be seen in Table 1, karyotypes T-1586 and T-21 showed similar sensitivity to aberration induction by MH; the karyotypes do not differ from one another with respect to the location in time of the aberration maximum (43.3 and 48.5% at 21 h after treatment with mutagen). There were also no significant differences in the frequency of aberrations at a particular recovery time. Similarly, the action of this agent in both T-1586 and T-21 was of a delayed type; only chromatid aberrations in the first metaphase after treatment was observed.

Figure 1 shows that pattern of intrachromosomal distribution of MH-induced aberrations in karyotype T-1586. What is remarkable in these data is that the localized breakage effect of MH proved to be nearly the same as that of mitomycin C (Gecheff 1989). Again, segments 47 (chromosome 7) and 39 (chromosome 6) appear to be clearly pronounced aberration "hot spots"; 23.3 and 13.7%, respectively, of all aberration breakpoints are found to be localized in these segments. As shown in Fig. 1, this effect is mostly expressed for duplication-deletions and especially for intercalary deletions; the percentage involvements in these aberration types of segment 39 are 16% for each type and those of segment 47 are 27 and 23%, respectively. The similarity of intrachromosomal distribution patterns of MH- and mitomycin-C-induced aberrations also applies in this case to some other segments that show very low expressivity of their aberration "hot spots". Most of these segments (10, 20, 26, 28, 18, 40, 42, 48, and 50) are known to contain heterochromatin, as defined by Giemsa-banding (Gecheff 1989).

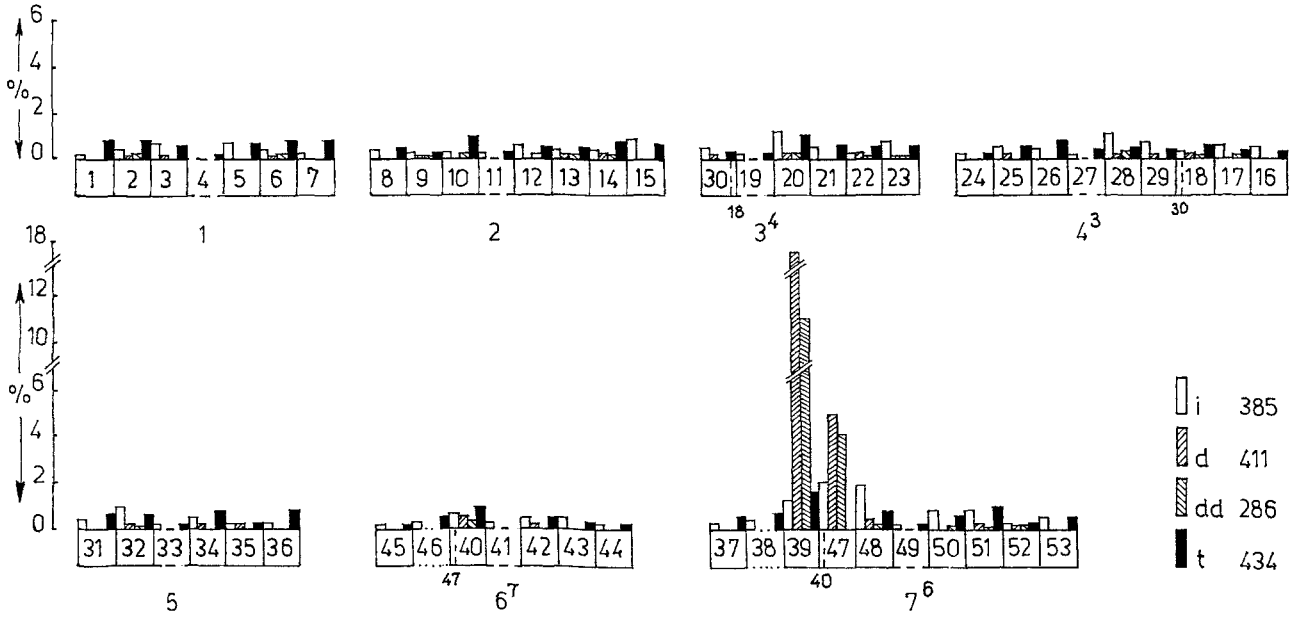

Fig. 2. The distribution of MH-induced chromatid aberrations (*i* isochromatid breaks, *d* intercalary deletions, *dd* duplication-deletions, *t* chromatid translocations) along the chromosomes of karyotype T-21

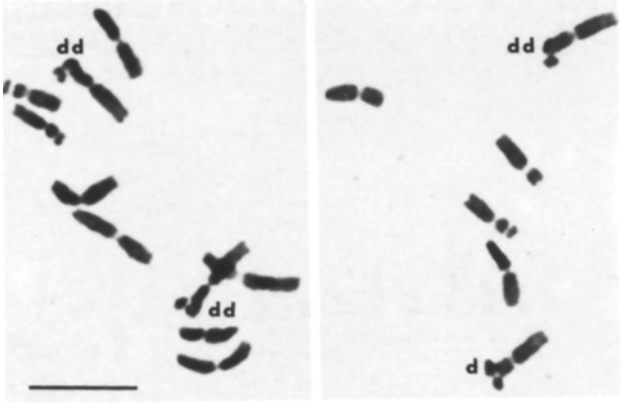

Fig. 3. Portions of two metaphase cells showing simultaneous involvements in intercalary deletions (*d*) or duplication-deletions (*dd*) of both homologous segments 39. Bar represents 10 μm

Figure 2 presents the localization of MH-induced chromatid aberrations in karyotype T-21, marked by the tandem position of the two most sensitive segments, 39 and 47, in the reconstructed chromosome 7^6. Interestingly enough, there were many findings in common in the overall response of the karyotype and the reaction of segments 39 and 47 in particular, on the one hand, and those observed in mitomycin C experiments, on the other. These segments again appear to be the most pronounced aberration "hot spots", intercalary deletions and duplication-deletions being the most frequently induced aberration types. Similarly, the relative involvement in aberrations of segment 39 in the new position (karyotype T-21) dramatically increased (31.4% of all aberration break-points) compared to its involvement in aberrations in the standard position (karyotype T-1586). A main reason for this response again seems to be the increased predilection of this segment to form intercalary deletions (approx. 50% of all aberrations of this type, versus 16% in the case of T-1586) and duplication-deletions (45 versus 16%).

Another important observation confirmed in this study is that, in most cases (ca. 78%), the specific constitution of chromosome 7^6 in karyotype T-21 gives rise to a clear-cut increase in the size of the chromosome segment which is involved in chemically induced intercalary deletions and duplication-deletions. In the standard position of segments 39 and 47, nearly all MH-induced aberrations of these types involve very small regions (sometimes probably below the resolution power of the light microscope), situated next to the secondary constrictions of chromosomes 6 and 7. In contrast, the size of the chromosome region involved in the aberration types mentioned above varies over a wide range, but most often embraces the whole of segment 39, or even exceeds its size, involving a part of the next segment 47. An important indication of this high position-dependent effect of MH is that, occasionally, both homologous regions of chromosome 7^6 in the same cell were affected (Fig. 3).

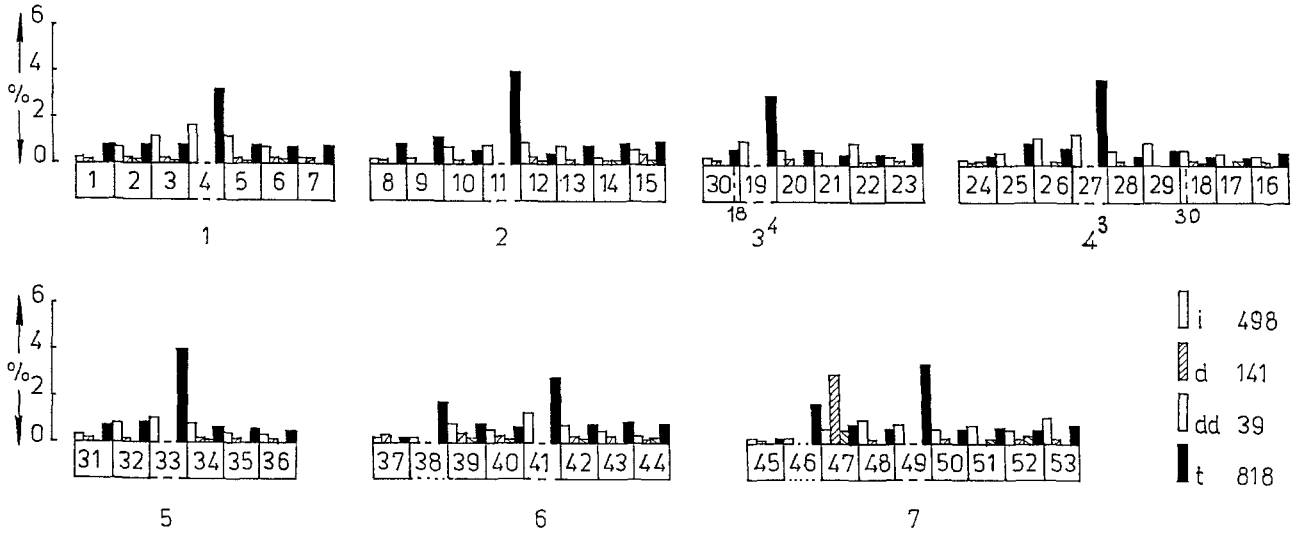

Fig. 4. The distribution of EMS-induced chromatid aberrations (*i* isochromatid breaks, *d* intercalary deletions, *dd* duplication-deletions, *t* chromatid translocations) along the chromosomes of karyotype T-1586

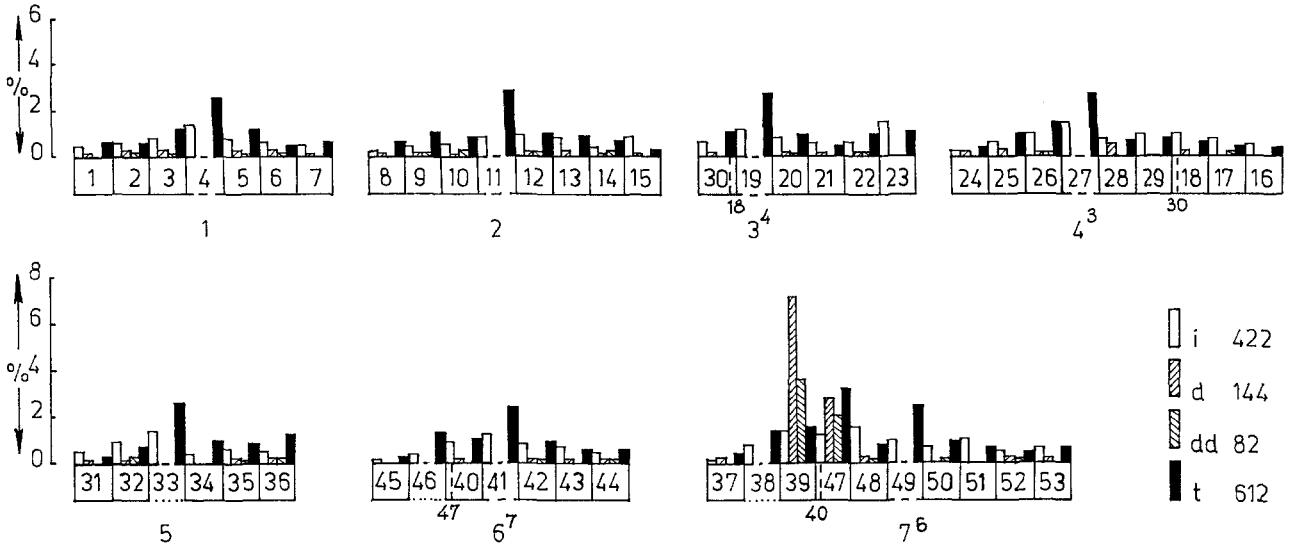

Fig. 5. The distribution of EMS-induced chromatid aberrations (*i* isochromatid breaks, *d* intercalary deletions, *dd* duplication-deletions, *t* chromatid translocations) along the chromosome of karyotype T-21

Frequency and intrachromosomal distribution of aberrations induced by EMS

The frequency of chromatid aberrations at different recovery times after treatment with EMS of both T-1586 and T-21 is presented in Table 1. It is obvious that, as in the case of MH, there is no significant difference in the sensitivity of the karyotypes tested to the action of EMS.

One of the most remarkable features of the distribution patterns of EMS-induced chromatid aberrations in both T-1586 (Fig. 4) and T-21 (Fig. 5) is that the centromeres of chromosomes (segments 4, 11, 19, 27, 33, 41, and 49) proved to be the most pronounced aberration "hot spots". It should be noted that only a few, if any, MH-induced isochromatid breaks and chromatid translocations were found to be localized in these regions. Moreover, in comparison with MH, the activity of EMS in segments 39 and 47, when they are in standard position (see Fig. 4), is very low; in fact, only the involvement of segment 47 in intercalary deletions surpasses the confidence limit for random aberration distribution.

The most important feature to be seen in Fig. 5 is that both segments 47 and 39, when combined by reciprocal translocation in tandem position in chromosome 7^6, re-

spond to EMS treatment with clearly pronounced aberration clustering. What is remarkable in this case is that segment 39, which is nearly "silent" in the standard position (Fig. 4), turns into the most pronounced aberration "hot spot" in karyotype T-21 (approx. 21% of all aberration break-points were localized in this segment). Similar to the results of experiments with mitomycin C and MH, the increased sensitivity of segments 39 and 47 to the action of EMS was not accompanied by any alteration in the overall sensitivity of the karyotype. As expected, the size of the chromosome region involved in EMS-induced intercalary deletions and duplication-deletions in segment 39 of chromosome 7^6 again proved to be significantly increased.

Discussion

Among the multitude of experimental data concerning the regional specificity of chemical mutagens, there are examples of an extremely pronounced clustering of induced chromosome damage. This response of chromosome structures to the action of mutagens proved to be (1) agent-specific and (2) position-specific, i.e., it may depend on the nature of the mutagenic factors applied (Schubert and Rieger 1977) or may be directly influenced by the position in the karyotype of the potential "hot spot" segment or by the structural features of the karyotype as a whole (Rieger and Michaelis 1972; Schubert et al. 1979).

The results described in this study, which is an immediate continuation of our earlier investigations on the position-specific effects of chemical mutagens (Gecheff 1989), provide further convincing evidence of the decisive role that chromosome constitution plays in the processes underlying the mutagenic specificity at the chromosome level. The most important conclusion to be drawn from these experiments is that segments 39 and 47, situated next to the secondary constrictions of standard chromosomes 6 and 7 of karyotype T-1586, when tandemly combined by reciprocal translocation in chromosome 7^6 of karyotype T-21, behave in a rather similar way. Their behavior is independent of the nature of the chemical mutagens applied, as far as the distribution pattern of induced aberrations is concerned. This phenomenon may be characterized as follows.

1. These segments, which contain heterochromatin as indicated by Giemsa-banding (Georgiev et al. 1985), when tandemly combined in reconstructed chromosome 7^6 of karyotype T-21, appeared to be the most pronounced aberration "hot spots", the expressivity in segment 39 always being higher. This tendency was observed even in the case of EMS treatment, where segment 39 in the standard position did not show increased sensitivity.

2. The pronounced "hot spot" character of these segments proved to be mainly due to their preferential involvement in intercalary deletions and duplication-deletions. Similar results were reported earlier both in barley and *Vicia faba* (Nicoloff et al. 1979; Schubert et al. 1981). However, the rate of these aberration types in segment 39 of karyotype T-21, after treatment with MH, was so high that this agent might actually be used for a purposeful reconstruction of the barley genome.

3. The most striking feature of this position-specific effect concerns the size of the chromosome segment involved in the induced intercalary deletions and duplication-deletions. Independent of the chemical mutagen applied, the specific constitution of chromosome 7^6 in the majority of cases resulted in a marked increase of the region involved in these aberration types in segment 39. The possible mechanisms underlying this phenomenon were discussed in a previous paper (Gecheff 1989) and the results obtained here support our earlier assumptions.

The results obtained confirm some of the former inferences of Schubert et al. (1985) concerning relationships between chromosome/karyotype constitution and mutagen sensitivity in *Vicia faba*. Thus, comparative analysis of the overall and differential sensitivity of the two karyotype variants showed that increased sensitivity of certain individual "hot spot" segments was usually compensated for by decreased sensitivity of other segments. For instance, in the case of EMS treatments, the increased sensitivity of segments 39 and 47 in karyotype T-21 was accompanied by decreased involvement in aberrations of the centromere regions, the last again being clearly pronounced aberration "hot spots". As a result of such compensatory effects, no difference in the overall sensitivity of the karyotypes used was observed.

Furthermore, as has been established in *Vicia faba* (Rieger et al. 1977), no correlation between the mode of action of the mutagens and the corresponding distribution patterns of chromatid structural changes was established. Chemical mutagens such as MH and mitomycin C, each probably inducing specific primary lesions in DNA, produced nearly the same patterns of chromosomal aberration distribution in both karyotype T-1586 and T-21.

Because of the lack of extensive knowledge of the molecular mechanisms involved in the processes of chromosome aberration induction, any detailed interpretation of the position-specific effects found in this study will contain many speculative elements. A successful attempt to comprehensively explain some of these effects was made by Kaina et al. (1979), but it seems most unlikely that the mechanisms proposed are unique. The present study is one of the few examples of true position effect in the expression of chemically induced structural mutations at the chromosome level, revealing some new aspects of this phenomenon.

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