The Effect of Kinetin, Gibberellic Acid and Indole Acetic Acid on EMS-Induced Somatic Mutation and Recombination in *Drosophila melanogaster*

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Received: 28.01.1999

**Abstract:** The effect of plant growth hormones (kinetin, gibberellic acid (GA₃) and indole acetic acid (IAA)) on EMS-induced mutant wing spots was studied with the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. GA₃ reduced all kinds of EMS-induced spot. While a 10⁻³ M concentration of kinetin reduced only the number of EMS-induced twin spots, a 10⁻⁴ M concentration was seen to increase the number of all types of spot. The same concentrations of IAA gave variable results. A 10⁻⁴ M concentration of IAA caused a decrease only in the number of large single spots. Our results confirmed that plant growth hormones, especially GA₃, can act as a bio-antimutagen.

**Key Words:** Ethyl Methanesulphonate, Gibberellic Acid, Indole Acetic Acid, Kinetin, Antimutagenicity, Somatic Mutation and Recombination Test, *Drosophila melanogaster*.

**Introduction**

Various types of inhibitor and suppressor against mutagens and carcinogens are found in several organisms and environments. Two main chemical groups of inhibitors against various
mutagens have been identified, based on their modes of action: desmutagens, which inactivate the chemical mutagen; and bio-antimutagens, which activate the repair system until fixation of the mutation occurs (1-3). Many antimutagens were discovered by bacterial tests such as the Ames test. Cancer depression tests are also required, but those using mammals require too much time and expense. The wing spot test with *Drosophila melanogaster* has become useful as a second screening method for mutagens between bacterial and mammalian systems. *Drosophila* is a higher organism than bacteria, being more similar to mammals with its chromosomes and P450 enzymes (4).

There are some reports on the antimutagenic effect of plant growth hormones in plants. It was reported that plant growth hormones reduced the mutagenesis caused by gamma rays, X-rays, EMS (Ethyl Methanesulfonate) and sodium azide (NaN₃) (5-10). The mechanism of antimutagenic effect has yet to be elucidated. This study was carried out in order to determine whether plant growth hormones were effective in a similar fashion when applied to animals.

Thus, the suppressing effect of plant growth hormones (kinetin, gibberellic acid (GA₃) and indole acetic acid (IAA)) on EMS-induced mutant wing spots in the SMART assay of *Drosophila melanogaster* was tested. There have been several reports on the application of the wing spot test for bio-antimutagens (2, 11-13).

### Materials and Methods

1. **Chemicals**

   Drosophila instant medium was obtained from Ward’s Biology. Ethyl methanesulfonate (EMS) was from Merck (Durmstadt, Germany). Indole acetic acid (IAA), gibberellic acid (GA₃) and kinetin were obtained from the Sigma Chemical Co. (St. Louis, Mo, USA).

2. **Drosophila Stocks**

   We used two stocks of *D. melanogaster* for a cross of mwh males with flr³-ln(3LR)TM3;rp²sep bx⁷xe e Ser females (for a description of the markers see Lindsley and Zimm 1992) (14). These stocks were originally obtained from the laboratory of Ulrich Graf, Swiss Federal Institute of Technology (ETH), University of Zurich. Both mwh and flr³ recessive wing mutations are located on the left arm of chromosome 3. Homozygous mwh wing blade cells develop several tricomes per cell instead of the usual single one and homozygous flr³ cells produce deformed tricomes. Flies were reared at 25±1°C and 60% relative humidity on cornmeal-agar medium (15).

3. **Experimental Procedures**

   Eggs from crosses between the two strains mentioned above were collected during an 8 h period. The larvae were allowed to grow to the 3rd instar for 72±4 h and were transferred to a culture bottle which contained cellulose powder and EMS (50 mM), to which they were exposed for 6 h. The EMS-treated larvae were counted and moved to an instant medium which contained a plant growth hormone. To test the mutagenicity, the mutagen-treated larvae were
transferred to an instant medium without a plant growth hormone. Emerged adult flies of trans-heterozygous (mwh/flr3) were stored in 70% ethanol. Their wings were mounted in Faure’s solution and examined under 500x magnification for the presence of mosaic spots. The number of spots as well as their types and size were recorded. The observed wing hair spots were classified as small single spots (SSS), large single spots (LSS) or twin spots (TS) according to the method of Graf et al. (4). One or two mwh or flr mutant cells were scored as small single spots. Three or more mwh or flr mutant cells were scored as large single spots. Neighboring mwh and flr mutant cells were scored as twin spots. The \( \chi^2 \) -test was performed on the number of spots per wing for each plant growth hormone-treated group and the positive control (mutagen only) (16).

**Results and Discussion**

EMS is an alkylating agent which is able to produce point mutations and small deletions as well as chromosome breakage; its recombinogenic activity is also known (17, 18). First, the mutagenicity of EMS was investigated. In this study all kinds of spot (SSS, LSS and TS) were seen to be increased with EMS at 50 mM (Table 1). This concentration of EMS was used throughout the experiments.

The antimutagenic effects of plant growth hormones (kinetin, GA3 and IAA) tested on EMS-induced mutation, are shown in Table 1 and Fig. 1. Three types of spot are considered to be the result of gene mutations and chromosome aberrations in SMART (4, 19, 20). Small single spot and large single spot are due to gene mutations, chromosome deletion, non-disjunction, or mitotic recombination. The twin spot is due exclusively to mitotic recombination (4). In this study, 10^{-4} M kinetin had no ability to decrease the mutagenicity of EMS. However, a significant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of wings</th>
<th>Small single spots</th>
<th>Large single spots</th>
<th>Twin spots</th>
<th>Total spots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (water)</td>
<td>200</td>
<td>0.04*</td>
<td>0.02*</td>
<td>0.01*</td>
<td>0.06*</td>
</tr>
<tr>
<td>Control (EMS)</td>
<td>253</td>
<td>0.23</td>
<td>0.45</td>
<td>0.17</td>
<td>0.85</td>
</tr>
<tr>
<td>10^{-4} M Kinetin</td>
<td>165</td>
<td>0.33’</td>
<td>0.82’</td>
<td>0.20</td>
<td>1.35’</td>
</tr>
<tr>
<td>10^{-3} M Kinetin</td>
<td>88</td>
<td>0.20</td>
<td>0.38</td>
<td>0.08’</td>
<td>0.65</td>
</tr>
<tr>
<td>10^{-4} M GA3</td>
<td>210</td>
<td>0.11’</td>
<td>0.40</td>
<td>0.01’</td>
<td>0.52’</td>
</tr>
<tr>
<td>10^{-3} M GA3</td>
<td>98</td>
<td>0.13’</td>
<td>0.27’</td>
<td>0.02’</td>
<td>0.42’</td>
</tr>
<tr>
<td>10^{-4} M IAA</td>
<td>89</td>
<td>0.13</td>
<td>0.29’</td>
<td>0.13</td>
<td>0.56’</td>
</tr>
<tr>
<td>10^{-3} M IAA</td>
<td>79</td>
<td>0.23</td>
<td>0.62’</td>
<td>0.13</td>
<td>0.97</td>
</tr>
</tbody>
</table>

* Significantly different from the control (EMS) at \( P<0.05 \) (\( \chi^2 \)-test).
increase in the number of SSS and TS was observed at the same concentration (P<0.05). A $10^{-3}$ M kinetin concentration decreased the number of TS (P<0.05). Also $10^{-4}$ M and $10^{-3}$ M GA$_3$ reduced the number of all types of spot induced by EMS. Therefore GA$_3$ might suppress the EMS-induced gen mutations, chromosome deletion, non-disjunction and mitotic recombination.

Figure 1. The effect of kinetin, GA$_3$ and IAA on mutations induced with EMS.
When IAA was used, the number of LSS decreased significantly at 10^{-4} M concentration, but an increase was observed with 10^{-3} M IAA concentration. The observation of an increase in the number of single spots of the 10^{-4}M kinetin and 10^{-3} M IAA groups may indicate a mutagenic effect of plant growth hormones. In future studies, studying the mutagenic effect of the plant growth hormones studied here and others will be important for an explanation of this phenomenon.

In some previous studies, it has been reported that some chromosomal abnormalities, resulting from EMS and gamma rays treatment, were corrected or at least the effect of those mutagens was decreased with the application of GA_{3} and IAA (6, 8-10). Here, it was confirmed that plant growth hormones, especially GA_{3}, were also an effective bio-antimutagen in animals.

References


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