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

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Abstract: The effect of plant growth hormones (kinetin, gibberellic acid (GA_3) and indole acetic acid (IAA)) on EMS-induced mutant wing spots was studied with the somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. GA_3 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 GA_3, can act as a bio-antimutagen.

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

Drosophila meanogaster'de EMS ile İndüklenmiş Somatik Mutasyon ve Rekombinasyon Üzerine Kinetin, Gibberellik Asit ve İndol Asetik Asitin Etkisi

Özet: Bu çalışmada, *Drosophila melanogaster*'in somatik mutasyon ve rekombinasyon testi kullanılarak EMS ile indüklenmiş mutant kanat benekleri üzerine bitki büyüme hormonlarının (kinetin, gibberellik asit (GA_3) ve indol asetik asit (IAA)) etkisi araştırıldı. GA_3, EMS ile indüklenen bütün kanat beneklerini azalttı. Kinetinin 10^{-3} M konsantrasyonu EMS ile indüklenen ikili beneklerin sayısını azaltırken, 10^{-4} M konsantrasyonu beneklerin bütün tiplerinin sayısında artışa neden oldu. Aynı konsantrasyonlardaki IAA ise değişken sonuçlar verdi. 10^{-4} M IAA yalnızca büyük tekli beneklerin sayısını indirgedi. Sonuçlarımız bitki büyüme hormonlarının (özellikle GA_3'ün) bio-antimutagen olabileceğini düşündürmektedir.

Anahtar Sözcükler: Etil Metansulfonat, Gibberellik Asit, Indol Asetik Asit, Kinetin, Antimutajenite, Somatic Mutasyon ve Rekombinasyon Testi, *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 (NaN_3) (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 (GA_3) 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), giberrellic acid (GA_3) 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 flr^3/In(3LR)TM3,$r^i p^p$sep bx^{34e} e^s 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 flr^3 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 flr^3 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/flr^3) 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 X^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 a 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, GA_3 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 gen 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 1. The effects of kinetin, GA_3 and IAA on the mutagenicity of EMS.

Treatment	No. of wings	No. of spots / wing			
		Small single spots	Large single spots	Twin spots	Total spots
Control (water)	200	0.04*	0.02*	0.01*	0.06*
Control (EMS)	253	0.23	0.45	0.17	0.85
10^{-4} M Kinetin	165	0.33*	0.82*	0.20	1.35*
10^{-3} M Kinetin	88	0.20	0.38	0.08*	0.65
10^{-4} M GA_3	210	0.11*	0.40	0.01*	0.52*
10^{-3} M GA_3	98	0.13*	0.27*	0.02*	0.42*
10^{-4} M IAA	89	0.13	0.29*	0.13	0.56*
10^{-3} M IAA	79	0.23	0.62*	0.13	0.97

* Significantly different from the control (EMS) at $P< 0.05$ (x^2-test).

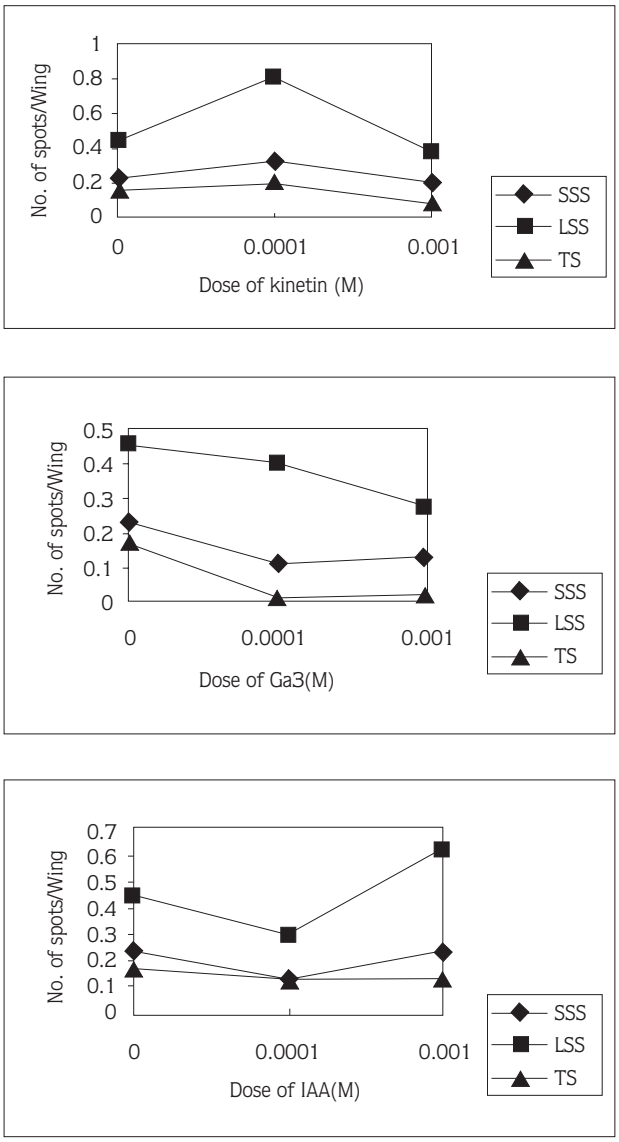

Figure 1. The effect of kinetin, GA_3 and IAA on mutations induced with EMS.

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.

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.

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