Gamma Field Symposia

Number 5

MUTATIONS INDUCED BY RI AND CHEMICALS

1966

THE INSTITUTE OF RADIATION BREEDING
MINISTRY OF AGRICULTURE AND FORESTRY
Ohmiya-machi, Ibaraki-ken
Japan
MUTATIONS INDUCED
BY
RI AND CHEMICALS

Report of Symposium
held on
July 27 to 28, 1966

Institute of Radiation Breeding
Ministry of Agriculture and Forestry
Ohmiya-machi, Ibaraki-ken
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FOREWORD

Numerous investigators tried to induce mutations in many different organisms by physical and chemical agents of the various nature, and at the present time it has been shown that virtually all morphological and physiological characters of plants can be changed by induced mutations. This indicates that there appears to be no doubt about the future importance of induced mutations in plant breeding. However, it is necessary to increase the total yield of mutations and to find the differences about the mutation spectrum produced by the various types of radiations and by various chemical mutagens.

In view of this, biophysicists, geneticists and plant breeders were brought together to explore and discuss some of the more recent findings and interpretations concerning the induced mutations by means of internal treatment with radioisotopes and chemical agents.

We wish to express our appreciation to members of the organizing committee, to the speakers and the chairmen.

Previous symposia in this series are:

1962—Radiation Injury and Somatic Mutation
1964—Cell Differentiation and Somatic Mutation
1964—Mutations in Quantitative Traits
1965—The Use of Spontaneous Mutations in Plant Breeding

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Chairman  T. Kobayashi

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Efficiency of mutation induction by the absorption
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Induction of mutants in sweet potato by absorption of $^{32}$P ..............K. Sawai

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E. Yamaguchi and
E. Inagaki

Chairman  H. Yamaguchi

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Chairman  K. Osone

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floral development, gametogenesis and embryogenesis under
chronic gamma irradiation ........................................................K. Yamakawa
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COMPARATIVE STUDIES OF MUTATION FREQUENCIES INDUCED BY $^{32}$P TREATMENT AND GAMMA-IRRADIATION IN THE SILKWORM

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INTRODUCTION

When a $^{32}$P atom disintegrates, a $\beta$-particle of average energy 0.7 MeV is emitted, and the nucleus of the sulfur atom produced has an average recoil energy of about 30 eV. Thus the mutagenic effect of decay of $^{32}$P incorporated into the constituents of a living organism could be due either to the effects of the $\beta$-emission accompanying radioactive decay or to some noxious event engendered by the transmutated atom itself, such as chemical valence change.

Several workers have reported pronounced mutagenic effects of $^{32}$P incorporated into DNA in bacteria as reviewed by Stent and Fuerst (1960). For example, Kaudewitz et al. (1958) showed that the frequency of auxotrophic mutants found in $^{32}$P labeled cells of Escherichia coli B/r is about fifty times or more higher than that in an external radiation control culture with $^{32}$P added to produce the same ambient radiation intensity. Although $\beta$-ray effects cannot be ruled out, as pointed out by Strauss (1958) and Stent and Fuerst (1960), it may be concluded that the mutagenic action of $^{32}$P incorporated into bacterial DNA is, at least partly, due to the effect of transmutation of incorporated $^{32}$P atoms.

Similar studies with higher organisms seem to show no parallel effects to those with microorganisms. Numerous reports have been published, and reviewed by Oftedal (1959), on the overall effects of $^{32}$P on mutation induction, especially with Drosophila. However, because of the physiological complexity of spermatogenesis of this insect as well as its geometrical complexity the results obtained with Drosophila are less conclusive concerning the mutagenic effect of $^{32}$P transmutation.

The present article reviews experiments with the silkworm of the past three years in our laboratory, some of which have not yet been published.

In order to determine the effect of $^{32}$P transmutation it is essential to recognize the contribution of $\beta$-emission accompanying the transmutation. In the present experiments with the silkworm, a group was treated with $^{32}$P, and another group

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was given a daily dose of gamma-rays varied day by day so as to match the change in the absorbed $\beta$-ray dose in the $^{32}$P group. Special care was taken in determining the absorbed dose in the gonad due to $\beta$-rays from the internal $^{32}$P. The mutagenic contribution of the $\beta$-rays was estimated by multiplying the daily mutation rate per rad of external irradiation (Tazima 1961) by the daily gonad dose due to the ingested $^{32}$P.

Excellent agreement in induced mutation frequency was found between the $^{32}$P treated and the gamma-rayed groups at the same absorbed dose. Using the incorporation data of $^{32}$P into DNA and other cell constituents of germ cells we shall discuss the absence of detectable transmutation effect in the present experiments.

I. Experiments with Male Silkworm

Materials and methods

Strain of silkworm. A wild type strain (C 108), and a marker strain homozygous for pe (pink egg, V-0.0) and re (red egg, V-31.7) were used.

$^{32}$P feeding method. The wild strain of silkworms was allowed to feed on $^{32}$P-coated mulberry leaves for about 6 hr on the third day of the fourth instar (15 days after hatching) when the majority of spermatocytes are just before meiosis (Sado, 1961) and maximal $^{32}$P incorporation into DNA of spermatocytes was expected. To prepare the labeled leaves 0.05 ml neutralized carrier-free $^{32}$Pi (The Radiochemical Centre, England) solution, having various specific activities from 3 $\mu$Ci/ml to 1 mCi/ml, was spread by a glass-rod on each fresh mulberry leaf over about 5 cm$^2$ area. The solutions on the leaves dried naturally in about two hours.

After feeding of $^{32}$P coated leaf for 6 hr, all the silkworms were transferred to fresh non-radioactive leaves and classified into 7 subgroups according to the specific activities of the $^{32}$P solutions given. Unfortunately, some silkworms did not eat all the $^{32}$P-coated leaf parts; therefore all the silkworms were measured as to their radioactivity individually with a Geiger-Müller counter at the middle of the pupal stage (34 days after hatching) and re-classified into 6 subgroups.

Gamma-ray irradiation. Silkworms not treated with $^{32}$P were exposed fractionally, i.e. day by day, to gamma-rays from $^{137}$Cs (dose rate: 224 R/hr) at room temperature. Three different daily doses were used: 20, 100 and 300 R on the first day when $^{32}$P was fed to the other silkworms. From the following day on, decreased doses of gamma-rays were given in parallel to the decreasing daily doses of $^{32}$P. Throughout the series of experiments, gamma-ray irradiation was carried out in normal rearing conditions.
Determinations of the absolute radioactivity. In a separate series of experiments, 70 ³²P-ingested individuals were reared and numbered separately. Each was weighed every day with respect to its body and excreta, and their radioactivities were measured. Four or five silkworms every day were ignited and their radioactivities were recounted in order to determine the selfabsorption coefficient. The ignition was carried out for one hour in electric furnaces at about 600°C.

Estimation of mutation rate. The specific locus method was used, with the egg color mutants, pe and re (Tazima, 1964). Treated individuals of the wild-type strain were mated to partners of the marker strain. The examination of mutant eggs was carried out under a binocular microscope 50-60 days after the eggs were laid. The number of detected mutants was pooled within each subgroup in order to calculate the over-all mutation frequency at the given dose.

Results

Daily doses of ³²P β-rays. The daily absorbed dose averaged over the whole body of a silkworm labeled with ³²P is simply proportional to the average specific activity C (µCi/g). The C(t) on the t-th day is given by:

\[ C(t) = \frac{C(1)}{M(t)} \quad (t \geq 2) \]

\[ C(1) = 0.63 \times \frac{C(1)}{M(1)} \]

where M(t) is the body weight of a larva on the t-th day. Derivation of Eqn.1b is based on the following reasoning. Since the whole body radioactivity changed markedly with time during the first day because of rapid uptake and excretion of ³²P, the average activity per body, \( \bar{C}(1) \), on the first day was estimated numerically from the activity variation curve: we obtained \( \bar{C}(1) = 0.63 C(1) \), where C(1) is the maximal activity on the first day.

Calculation of gonad dose for ³²P treatment. We assume that the gonad is a spherical tissue of radius b uniformly labeled with ³²P at the specific activity of c₀ and surrounded by a uniformly labeled infinite medium of specific activity c, and that the ratio of c₀ to c and b is constant during the developmental stage during exposure to ³²P β-rays. Under the above assumption, the gonad dose X(rad) for n days exposure can be written from Loëvinger's (Loëvinger et al. 1956) empirical equations as follows:

\[ X = \sum_{t=1}^{n} x(t) = f_β c_0 \beta(t) \]

\[ f_β = 51.2 E_β(1 + \frac{1}{4} \frac{c_0 - c}{c} \nu b) \quad (\nu b \leq 1) \]
where \( x(t) \) is the gonad dose rate (rad/day); \( c(1) \) is the average specific activity of the whole body on the first day; \( f_b \) is the conversion factor from \( c(1) \) (\( \mu \)Ci/g) to \( x(1) \) (rad); \( \beta(t) \) is equivalent to \( c(t)/c(1) \) which was determined experimentally by ignition data; \( E_\beta \) is the average energy of \( ^{32}\text{P} \) \( \beta \)-particles (0.694 MeV); \( \nu \) is the effective absorption coefficient of \( ^{32}\text{P} \) \( \beta \)-particles (9.2 cm\(^2\)/g).

The second term in the parenthesis of Eqn.2b derived from Loewinger's equation is a correction factor for gonad dose due to excess specific activity of gonad over the whole body. The measured value of \( b \) in the fifth instar was about 0.107 (g/cm\(^2\)) for testis and actual values of \( c \) and \( c_\beta \) are shown in Table I with relevant data. Hence we obtain from Eqn. 2b, the \( f_b \) value 67.

### Table I. The inhomogeneous distribution of \( ^{32}\text{P} \) in the male silkworm larvae on the third day of fifth instar (6 days after \( ^{32}\text{P} \) feeding)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Weight (mg)</th>
<th>Activity* (cpm)</th>
<th>Specific activity (cpm/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malpighian tubule</td>
<td>7.5</td>
<td>25</td>
<td>3.3</td>
</tr>
<tr>
<td>Testis</td>
<td>5.1</td>
<td>20</td>
<td>3.92</td>
</tr>
<tr>
<td>Fat body</td>
<td>225</td>
<td>137</td>
<td>0.609</td>
</tr>
<tr>
<td>Body fluid</td>
<td>110</td>
<td>50</td>
<td>0.455</td>
</tr>
<tr>
<td>Silk gland</td>
<td>678</td>
<td>690</td>
<td>1.02</td>
</tr>
<tr>
<td>Intestine</td>
<td>705</td>
<td>487</td>
<td>0.691</td>
</tr>
<tr>
<td>Epidermis</td>
<td>649</td>
<td>627</td>
<td>0.966</td>
</tr>
<tr>
<td>Whole body</td>
<td>2380</td>
<td>2035</td>
<td>0.855</td>
</tr>
</tbody>
</table>

* Counting efficiency of the G-M counter used was 1.5 per cent.

**Conversion factor from \( ^{32}\text{P} \) activity to gamma dose.** The method of calculating conversion factor from \( ^{32}\text{P} \) activity fed per head to gamma-ray exposure dose is described in detail in previous paper (Ikenaga and Kondo, 1965), so only the result of calculation will be shown.

For technical reasons, the specific activity of individual larva was measured during the pupal stage. The correlation between the apparent activity \( N_p \) (counts/min per head) at the pupal stage and the initial \( ^{32}\text{P} \) activity \( C(1) \) (\( \mu \)Ci/head) was determined as mentioned in Materials and Methods. The relation of \( N_p \) with \( C(1) \) was simply linear and the precise relation can be written as follows:

\[
\frac{C(1)}{N_p} = 8.4 \times 10^{-4} (\mu \text{Ci/counts/min}) \quad \text{(3)}
\]

Thus, we obtain the conversion factor from \( N_p \) (counts/min per head) to gamma dose \( (R) \) as follows:
\[ f \text{counts/min} = 4.0 \times 10^{-9} \text{(R/counts/min per head)} \quad \ldots \ldots \quad (4) \]

*Mutation frequency versus dose for $^{32}P$ and gamma treatments.* The mutation frequency at a given dose is calculated by dividing the sum of the numbers of $pe$, $re$ and $re$-mosaic mutants within each subgroup by the total number of its viable eggs. We have excluded $pe$-mosaic mutants because they were difficult to be identified.

We find from Eqn. 4 that male larvae irradiated with 20 R on the first day followed by decreased daily doses must have received the same total dose of 160 rad as those of $^{32}P$ fed larvae which possessed the $N_p$ value of 500 (counts/min per pupa). Carrying out a similar calculation for other gamma-rayed sub-groups, we can plot the mutation frequencies of the gamma-rayed group on the same scale as those of the $^{32}P$ group as shown in Fig. 1.

![Mutation frequency versus dose curve for $^{32}P$-fed and \( \gamma \)-rayed male silkworm. The total dose given to the silkworm with 20 R of \( \gamma \)-rays on the first day followed by decreasing daily dose is equal to the $^{32}P$ \( \beta \)-ray dose given to the male larva having 500 counts/min at pupal stage (see text).](image)

From Fig. 1 we find that the induced mutation frequency increased with dose both for $^{32}P$ and gamma treatments, and there was no significant difference in mutation frequency at the equivalent dose between the two treatments.

*Incorporation of $^{32}P$ into DNA.* Materials were prepared by homogenizing the copulatory pouches of five female moths each mated with a radioactive male
Table II. Absolute activities of $^{32}$P in sperm and their fractionated components

<table>
<thead>
<tr>
<th>Materials*</th>
<th>$^{32}$P activity** (dpm)</th>
<th>Weight *** (mg)</th>
<th>Specific activity *** (dpm/mg)</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>1,300,000</td>
<td>1,300</td>
<td>$1.0 \times 10^{4}$</td>
<td>1.0</td>
</tr>
<tr>
<td>Seminal fluid</td>
<td>5,770</td>
<td>5.0</td>
<td>$1.2 \times 10^{5}$</td>
<td>1.2</td>
</tr>
<tr>
<td>DNA</td>
<td>52</td>
<td>$2.8 \times 10^{-3}$</td>
<td>$1.9 \times 10^{4}$</td>
<td>19</td>
</tr>
<tr>
<td>RNA</td>
<td>870</td>
<td>$3.5 \times 10^{-3}$</td>
<td>$2.5 \times 10^{4}$</td>
<td>250</td>
</tr>
<tr>
<td>Protein</td>
<td>63</td>
<td>$3.0 \times 10^{-2}$</td>
<td>$2.1 \times 10^{4}$</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* The silkworm pupae were assayed 19 days after $^{32}$P feeding; five radioactive males used had the pupal activity in the range 3000 to 7000 cpm. The figures given for activity and weight are the values obtained by dividing the total values with five.

** Corrections were made for physical decay of $^{32}$P and counting efficiency of the radiation counter.

*** Because of some contamination with non-radioactive materials from the copulatory pouch of the female mated with the radioactive male, the figures in column 3 and 4 are somewhat over-estimates and under-estimates, respectively, and hence those in column 5 are subject to some systematic errors.

moth whose activity was in the range of 5000±2000 counts/min, and DNA, RNA and protein were extracted by the Schmidt-Thannhauser method. Table II summarizes the results obtained. The specific activities of sperm DNA, sperm RNA, and the whole body were approximately in the ratio 20 to 250 to 1.

II. Experiments with Female Silkworm

Materials and methods.

Experimental set up and procedures are essentially the same as that used in the experiments with the male silkworm except for $^{32}$P treatment.

The wild strain pupae were injected in the ventral side of the abdomen with 0.1 ml solution of $^{32}$P in distilled water with varying specific activities from 3 μCi to 200 μCi per ml, at the nineth day after pupation (35 days after hatching).

The injection period chosen for this study is eight or nine days before the meiotic stages for female silkworm. Careful injection with a fine needle minimized the amounts of fluid which pupae expel. Radiation control groups of the wild type were injected with 0.1 ml of distilled water.

Results

Specific activity of gonad. Six pupae, for each $^{32}$P injected group, were dissected three days after $^{32}$P injection. Ovary and some other organs were weighed and their radioactivities were counted after ashing. Table III shows the average
Table III. Distribution of $^{32}$P in the female pupa at three days after $^{32}$P injection

<table>
<thead>
<tr>
<th>$^{32}$P activity injected</th>
<th>Materials</th>
<th>Radioactivity (cpm)</th>
<th>Fresh weight (mg)</th>
<th>Specific activity (cpm/mg)</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body fluid</td>
<td>7,242</td>
<td>293.4</td>
<td>25.6</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>Viscera</td>
<td>4,914</td>
<td>170.9</td>
<td>28.8</td>
<td>1.37</td>
</tr>
<tr>
<td>0.3$\mu$Ci</td>
<td>Epidermis</td>
<td>14,259</td>
<td>1,042.0</td>
<td>13.7</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>9,918</td>
<td>299.9</td>
<td>43.1</td>
<td>2.05</td>
</tr>
<tr>
<td></td>
<td>Whole body</td>
<td>36,333</td>
<td>1,726.2</td>
<td>21.0</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Body fluid</td>
<td>20,758</td>
<td>275.8</td>
<td>75.3</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>Viscera</td>
<td>16,050</td>
<td>181.6</td>
<td>88.4</td>
<td>1.27</td>
</tr>
<tr>
<td>1$\mu$Ci</td>
<td>Epidermis</td>
<td>34,798</td>
<td>823.1</td>
<td>42.3</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>35,556</td>
<td>258.1</td>
<td>138.0</td>
<td>1.98</td>
</tr>
<tr>
<td></td>
<td>Whole body</td>
<td>107,162</td>
<td>1,538.6</td>
<td>69.6</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Body fluid</td>
<td>93,128</td>
<td>271.3</td>
<td>343.3</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>Viscera</td>
<td>80,330</td>
<td>171.3</td>
<td>468.8</td>
<td>1.39</td>
</tr>
<tr>
<td>5$\mu$Ci</td>
<td>Epidermis</td>
<td>186,756</td>
<td>870.2</td>
<td>214.6</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Ovary</td>
<td>164,859</td>
<td>244.7</td>
<td>673.8</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>Whole body</td>
<td>525,073</td>
<td>1,557.5</td>
<td>337.1</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Distribution of $^{32}$P activity in female pupae injected three different doses of $^{32}$P. The specific activity of ovary was just two times higher than that averaged over whole pupal body.

*Calculation of $^{32}$P β-ray dose in gonad.* A correction factor for gonad dose in the second term in the parenthesis of Eqn.2b is somewhat changed, owing to large size of ovary in the present condition ($r b > 1$), and $f_\beta$ the conversion factor from $c(1)$ ($\mu$Ci/g) to $x(1)$ (rad) can be written as:

$$f_\beta = 51.2 E_\beta \left[ 1 + \frac{3}{4} \left( 1 - \frac{1}{3} (1 + r b) e^{4 b} \right) \frac{c_g - c}{c} \right] (r b > 1)$$  \hfill (5)

where $E_\beta$, $r$, $b$, $c_g$ and $c$ are all the same as that defined in Eqn.2b. Using the values of $c_g$ and $c$ listed in Table III and the measured value of $b$, ca.0.387 (g/cm²) for ovary, we obtain from Eqn.5, the $f_\beta$ value 59.2.

Fortunately, since the pupa is a closed system, it need not to consider daily variation of specific activity $c(t)$ due to biological uptake and excretion of $^{32}$P, except for physical decay. Hence, from Table III and Eqn.5, we obtain the desired gonad dose $D_\beta$ at the first day after 1$\mu$Ci of $^{32}$P injection in the following:

$$D_\beta = 37 \text{ (rad/day)}$$ \hfill (6)

*Mutation frequency versus dose for $^{32}$P and gamma-ray treatment.* The average
numbers of viable eggs observed are about seventy to eighty thousand per subgroup. The mutation frequency at a given dose is calculated by dividing the sum of the numbers of $pe$, $re$ and $re$-mosaic mutants within each subgroup with the total number of viable eggs within the subgroup.

In order to compare the efficiency of mutation induction between $^{32}\text{P}$ and gamma-ray treatments, mutation frequencies were plotted against the same scale of the gonad dose, in rad unit, given in the first day of both treatments as shown in Fig. 2. The most important finding is that, again, there is no significant difference in mutation frequencies at the equivalent dose between the $^{32}\text{P}$ and gamma-ray treatment for female silkworm over the dose range studied.

![Graph showing mutation frequency versus dose rate for $^{32}\text{P}$-injected and $\gamma$-rayed female silkworm. The dose rate (rad/day) is expressed as dose in the first day after $^{32}\text{P}$ injection.](image)

*Fig. 2. Mutation frequency versus dose curve for $^{32}\text{P}$-injected and $\gamma$-rayed female silkworm. The dose rate (rad/day) is expressed as dose in the first day after $^{32}\text{P}$ injection. ○: $^{32}\text{P}$ treatment, ●: $\gamma$-ray irradiation.*

*Incorporation of $^{32}\text{P}$ into DNA.* Ovaries were taken out from radioactive pupae three days after $^{32}\text{P}$ injection, and fractionated into DNA, RNA and protein. Table IV summarizes typical data of $^{32}\text{P}$ incorporation into several cell constituents. From Table III and IV, it was found that the specific activities of ovary DNA, RNA, whole ovary and pupal body are approximately in ratios of 300, 90, 2 and 1, respectively.
### Table IV. Radioactivity distributions of $^{32}$P in ovary and their fractionated components

<table>
<thead>
<tr>
<th>$^{32}$P activity injected</th>
<th>Cell constituents *</th>
<th>Weight (mg)</th>
<th>Radioactivity (cpm)</th>
<th>Per cent activity</th>
<th>Specific activity (cpm/mg)</th>
<th>Specific activity** (dmp/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 $\mu$Ci</td>
<td>Acid soluble</td>
<td>19,488</td>
<td>72.16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipid</td>
<td>2,343</td>
<td>8.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>3,899</td>
<td>13.60</td>
<td>940</td>
<td>$9.68 \times 10^3$</td>
<td>$2.90 \times 10^4$</td>
</tr>
<tr>
<td></td>
<td>DNA</td>
<td>0.437</td>
<td>4.57</td>
<td>$2.28 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>28.4</td>
<td>0.99</td>
<td>9.4</td>
<td>96.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole ovary</td>
<td>744.2</td>
<td>53.9</td>
<td>555</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 $\mu$Ci</td>
<td>Acid soluble</td>
<td>59,378</td>
<td>57,00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipid</td>
<td>10,308</td>
<td>9.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>4,179</td>
<td>25.89</td>
<td>$6.54 \times 10^3$</td>
<td>$6.64 \times 10^4$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA</td>
<td>0.304</td>
<td>6.46</td>
<td>$2.21 \times 10^4$</td>
<td>$2.28 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>31.5</td>
<td>0.75</td>
<td>24.7</td>
<td>254</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole ovary</td>
<td>1019</td>
<td>181.7</td>
<td>$1.87 \times 10^3$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 $\mu$Ci</td>
<td>Acid soluble</td>
<td>268,947</td>
<td>59.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipid</td>
<td>44,223</td>
<td>9.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RNA</td>
<td>3,872</td>
<td>24.32</td>
<td>$2.92 \times 10^4$</td>
<td>$3.01 \times 10^5$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DNA</td>
<td>0.242</td>
<td>5.91</td>
<td>$1.11 \times 10^5$</td>
<td>$1.14 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>35.4</td>
<td>0.70</td>
<td>90.2</td>
<td>929</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Whole ovary</td>
<td>1047</td>
<td>742</td>
<td>$7.65 \times 10^3$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Materials were obtained by dissecting radioactive pupae three days after $^{32}$P injection.

** correction were made for physical decay of $^{32}$P and counting efficiency of the radiation counter.

### DISCUSSION

The agreement in induced mutation frequency between $^{32}$P and gamma treatments both for male and female silkworm leads to the conclusion that transmutation from $^{32}$P to $^{32}$S and recoil of daughter nuclei cause no detectable effects on the mutation induced as compared with the effects of $\beta$-rays under the present experimental conditions.

The absence of any detectable mutagenic effect of $^{32}$P transmutation in the present study agrees with similar negative results of King (1953) and Bateman (1955) in *Drosophila*. Furthermore, very recently Lee et al. (1966) carried out elegant experiments with *Drosophila* in which the effect of $^{32}$P transmutation and recoil of the $^{32}$S nucleus had been separated from the mutagenic effect of accompanying $\beta$-radiation by storage of $^{32}$P labeled spermatozoa in unlabeled *Drosophila*.
females. Their result is that there was no significant change during storage in
the rate either of chromosome breakage or of sex-linked recessive lethals that
could be attributed to transmutation and recoil. Ofstedal (1957, 1959) observed
an excess $^{32}$P-induced mutation frequency above that induced by $\beta$-radiation from
incorporated $^{91}$Y, and concluded that recessive lethals arose at a rate of about 1
per 100 $^{32}$P transmutations. However, in *Drosophila*, it is difficult to determine
accurately the gonad dose and the $^{32}$P incorporation into gonad DNA because of
the small dimensions of the gonad and the whole body. For instance, the estimated
fraction of $^{32}$P $\beta$-ray energy absorbed by the gonad varies with the authors:
0.0955 (King, 1952), 0.02 (King, 1953), 0.08 (Bateman, 1955) and 0.058 (Ofstedal,
1959).

Hence, we can make no definite statement about the possibility of mutation
induced in *Drosophila* by $^{32}$P transmutation.

Silkworm was used in experiments reported here to obtain easily $^{32}$P $\beta$-ray
dose in gonads and incorporation data of $^{32}$P into DNA of germ cells, partly
because of large size of their gonads. It is significant that when the effects of
$\beta$-rays were subtracted, there was no increase in frequencies of egg color mutations
that could be attributed to $^{32}$P transmutation and recoil.

Under the assumption that the recoil and transmutation might produce changes
in genetic material, certain requirement must be met to demonstrate such genetic
effects in a given organism: The amount of ionization in gonad produced by $^{32}$P
$\beta$-particles originating from external and internal $^{32}$P must be negligible. If it is
not negligible, it had to be determined by experiment or by calculation.

Calculation using Loevinger's equation shows that while a silkworm gonad
absorbs roughly 0.2—0.3 of the energy of the average $^{32}$P $\beta$-rays, a virus or small
bacteria absorb only about $10^{-4}$—$10^{-5}$. Thus one would expect it to be far easier
to detect the genetic effects of transmutation in microorganisms than in higher
livings, because the transmutation effects in higher organisms, if any, may be
masked by the superior $\beta$-ray effects.

In the previous report (Ikenaga and Kondo, 1965), we suggested that the
critical experiment to detect transmutation effect requires that the specific activity
of DNA should be about $10^4$ times higher than the average activity of the whole
organism. The reason for this is that each $^{32}$P decay is accompanied by emission
of a $\beta$-particle of average energy about 0.7 MeV which produces the order of
$10^4$ primary ionizations. As seen from Table II and IV, it is very difficult to obtain
such condition for silkworm by $^{32}$P ingestion or injection used in the present study.

To explain the absence of any detectable mutagenic effect of $^{32}$P transmutation,
it must be added that the chromosomes of higher forms may have such structural
and functional stability that could tolerate or repair the initial effect of transmutation and recoil of $^{32}$P incorporated into DNA.

For technical reasons, in the present study silkworm larvae were given not continuous but daily fractional gamma-irradiation. Because the dose rate was low and the daily dose was small, we may assume that there would be no large difference in the mutation frequency between daily fractional and continuous irradiations, although the continuous irradiation as used by King (1954) with Drosophila is certainly preferable. But this assumption is open to question for future study.

**SUMMARY**

The frequency of mutation induced in the male silkworm fed with $^{32}$P on the third day of the fourth instar and induced in the females injected with $^{32}$P at middle pupal stage was compared with that induced by gamma-irradiation. The daily dose of gamma-ray was carefully adjusted day by day so as to fit the daily change in the absorbed dose delivered to the gonad by the internal $^{32}$P. The gonad dose for the $^{32}$P treated group was estimated by applying Loevinger's equation; the parameters involved in the equation were experimentally determined with special care for biological factors. Using the $^{32}$P dose estimates thus determined, we found that the mutation frequency was equal for the $^{32}$P and gamma-ray groups at the same absorbed dose. We conclude that there could be no chance for a mutation induced by $^{32}$P transmutation to show under the present conditions.

**References**

8. Loevinger R., E. M. Japha and G. L. Brownell (1956) Radiation field and their dosi-
山口 内部照射の場合に、たとえば、\(^{32}\text{P}\) は細胞内で **locality** をあらわすと思われます。したがってラドの計算の際に、細胞質と細胞核での **locality** の差についてはどのように考えるべきでしょうか。

池永 その時に最も問題になるのは \(^{32}\text{P}\) のエネルギーで、大ざっぱに言えば、水の中でベータ線がどれくらい走れるかということです。平均エネルギーであるまとめと、それは 0.7 MeV であって、また水中の飛程は 3 mm であるから、\(^{32}\text{P}\) に関する限り、細胞内での吸収線量が大きいことはまずないものと考えられます。もっとも、非常に大きい細胞では問題があるでしょうが、非常にエネルギーの小さい \(^{3}\text{H}\) などの場合には、精密な計算を要するものと思われます。

代谷 DNA の比放射能から計算すると、たとえばカイコの場合に、1分子に数ケぐらい \(^{32}\text{P}\) が入っているのか、あるいは数分子に1ケの \(^{32}\text{P}\) が入っているのか、どちらでしょうか。

池永 オートラジオグラフのデータとか、1細胞あたりの DNA 数のデータを持っていませんが、大ざっぱな推定をしますと、カイコの雄の実験では、最後に交代するまでに \(^{32}\text{P}\) の崩壊したものが1精子あたり約300ケであると考えられます。

申し訴えましたが、\(^{32}\text{P}\) をカイコに与える時には、桑の葉に塗りつけて食べさせていた。また、注射は溶液のままおこなっています。
EFFICIENCY OF MUTATION INDUCTION BY
THE ABSORPTION OF $\beta$-EMITTING RADIOISOTOPES
IN PLANTS

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It must be great interests in mutation breeding to increase mutation frequency
and to get some informations about their spectra which may be changed by the
different induction methods.

In this regard, mutation induction by the absorption of $\beta$-emitting radioiso-
topes (RI absorption), the internal irradiation, which characterized by the genetic
effects of atomic transmutation and localized actions in macro or micro level, and
irradiation with low dose rate for relatively long time in living tissue, seem to
have some particularities as compared with external radiations.

Many works have been reported about this method since around 1950. However,
there were somewhat different opinions among researchers in evaluation of this
method for mutation induction from the view point of plant breeding.

One of the reasons of disagreement may be due to the instability or poor
repeatability of the effects. For example, in the seed treatments with $^{32}$P or $^{35}$S,
lethal dose or mutation frequency does not always give the same result among
reporters who use the same material and radioisotope.

These fluctuations of effects seem to be due to the amounts of absorbed RI
which vary with the difference of treating condition, such as soaking time,
temperature at soaking, volume of RI solution and so on.

In this meeting, some technical problems concerning method of treatment in-
cluding both for seeds and growing plants will be discussed first and then the
efficiency of RI absorption as an method of induction of mutation in comparison
with that of X or $\gamma$ irradiation will be taken up, though it is a limited case as
chlorophyll mutation in rice.

1. Factors Affecting the Efficiency of Seed Treatment

   Physical and biological conditions.

   Soaking time seems to be the most important factor. The relation between
absorption rate of RI and soaking time in the treatment of $^{32}$P to the dormant
tomato seeds is shown in Fig. 1. In this figure, it is found that there is the re-
markable increase of absorption from 3 days to 5 days, and, therefore, it makes
the curve sigmoid. In another experiment, when germinated tomato seeds were soaked in $^{32}$P solution under the same conditions with the experiment above mentioned, absorption was very active from the beginning of soaking and absorption rate at 24 hr was 80 per cent which was almost the same level with that of 5 days soaking in dormant seeds.

These and another experimental evidences show that the initiation of active RI absorption must be almost to coincide with starting time of radicle elongation. Autoradiographic observation of maize embryos soaked in $^{32}$P or $^{35}$S solution clearly indicated that the incorporation of these RI to the meristematic tissue took place at first after root protrusion. This means that any effective internal irradiation in soaking of dormant seeds can not be expected before root protrusion.

According to these findings, it can generally be concluded about soaking time that (1) one week or more of soaking after root protrusion is necessary to give complete internal irradiation and (2) use of germinated or pre-soaked seeds may be available to shorten soaking time which is very important to avoid loss of treated seed by rotteness or death due to continuous submergence.

Absorption of RI by soaked seed is also influenced more or less by the soaking temperature, volume of RI solution and the kind of seed bed for soaking.

Some experimental results concerning those factors will be briefly presented as
follows.

Low temperature at soaking decreases absorbing activity. However, in case of soaking time it is long enough and temperature is not too low for germination and final absorption rate will come up to good level.

It has been suggested that there might be an optimum volume of RI solution in which seeds were soaked to keep sufficient absorption under proper soaking time. In the $^{32}$P treatment of rice, wheat and tomato, range of optimum volume of RI solution seemed to be 5-10 times of the volume of materials.

It has been postulated about the kind of seed bed, that soaking on some materials, such as filter paper and sand, brought more or less the decrease in final absorption rate compared with direct soaking. This reason might be thought that part of RI is absorbed on the surface of these materials and become unavailable for the roots. In case of $^{32}$P treatment of germinated barley seeds, it was found that the amount of $^{32}$P in the seedling come from the soaked seeds on the filter paper and sand were 70 per cent and 30 per cent of that of direct soaking, respectively.

In this case, however, loss of the treated seeds caused by rotness was very little and variation in growth was decreased.

*Individual variation of RI absorption.*

The amount of RI absorbed in each seedling after treatment is usually different one by one. Such an individual variation found in the $^{32}$P treatment of dormant rice seeds is shown in Fig. 2.

This must be caused obviously by the lack of uniformity of germination among treated seeds.

Gustafsson (1950) observed in the treatment of barley seeds that the maximum amount of absorption was 6 times of the minimum in case of $^{32}$P and 25 times in case of $^{35}$S for limited number of seedlings by sampling.

Hentrich (1960) also observed the same tendency in case of the $^{32}$P treatment of barley seeds and postulated that the individual variation of RI absorption was an important problem in the internal irradiation affecting its efficiency.

Such individual variation occurs in the treatments of both dormant and germinated seeds. And it is very difficult to avoid this disadvantage in the dormant seed treatment but when the germinated seeds are used, it is possible to reduce individual variation by using homogeneously germinated seeds. It is confirmed in the experiment of $^{32}$P treatments with two kinds of doses in barley and tomato. In this experiment, variation coefficients of $^{32}$P content among the selected seeds were less than half of that among the unselected seeds and maximum to minimum ratios were kept less than two in all treatments of selected seeds.
Fig. 2. Relation between length of embryo and amount of $^{32}$P in each seed in the dormant seed treatment of rice.

A special case of seed treatment. $^{32}$P treatment of phosphorus deficient seeds. Specific radioactivity in tissue or organs after treatment with RI is basically important for internal irradiation. It usually increases with the dosage, and also it may increase by reducing phosphorus level in material itself.

Biological effects in the latter case were examined by using phosphorus deficient rice seeds.

Three kinds of rice seed which are different in the phosphorus content, were soaked in $^{32}$P solution under the same condition.

Absorption rate did not change in these three kinds of materials.

In $R_1$ generation, it was found that low level in phosphorus content promoted
the physiological damages, especially the decrease of survival rates at seedling stage. However, much difference was not found among seed fertilities of these materials.

On the chlorophyll mutations observed in R2 generation, the material having the lowest phosphorus content gave very high mutation rate in all repeated experiments, and it was almost twice higher than that of normal seeds (Table 2).

These results suggest that the 32P treatment of such seeds being sufficiently low in phosphorus content has a possibility to increase mutation frequency.

2. Problems in the Treatment for Growing Cereals and its Effect

Method of treatment.

Water culture or sand culture with RI has generally been employed in the treatment for growing cereals.

The results of experiments, where rice plants were treated by 32P under water culture, showed that amounts of absorbed RI were mostly proportional to the

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**Fig. 3.** Distributions of RI in stalk and panicle in rice after injection at different internodes.
- Dose: 80μCi/stalk
- Stage of treatment: 8 days after heading
- ↑: Indicate the position of RI injection
given doses and their standard deviations among plants were very small in all cases. Therefore, water culture or sand culture seems to be the best way to treat the growing plants from the standpoint of accuracy. But, the trouble of handling must be very large when treatment is applied on a large scale.

Another method of treatment is to inject RI solution directly to some part in top of plant.

This injection method has been employed only in woody plant like fruit tree, but, in my experiences, it was often available for growing cereals. In particular, injection to the internode at various stages during reproductive growth of cereals seems to be quite reasonable method because of its simplicity of handling and relatively honest respondence to given doses.

However, the fact that some RI has poor movement in plant after injection and the amount of RI accumulated in particular organ varies depending on the position where injection was applied, will be very important in the application of this method.

An experimental result concerning with this problem in rice is shown in Fig. 3. The amount of RI in panicles varies markedly with the change of position of injection in $^{35}$S treatment, but it is not so in $^{32}$P treatment.

*Dose-effect relationship.*

Relation between the amount of RI accumulated in a certain part of plant, such as matured embryos and the given doses in reasonable range is found to be highly linear. This relation may differ from the results of the seed treatments in which the absorption rates decrease with the increase of dose as reported by some workers.

Expressions of biological effects are, actually, much more complicated by the combinations of doses and the developmental stages of plant to which the treatments are applied.

However, it was evident that physiological damages in treated generation were roughly proportional to given doses from the observations on seed fertility in the experiments where rice, wheat and barley were treated by $^{32}$P or $^{35}$S at a certain stage of their reproductive growth using internode injection method.

While, mutation frequencies found in R$_2$ generation seemed to be relatively good in response to the given doses.

The suitable doses (per stalk by internode injection) were roughly estimated from the experiment as follows:

Regarding $^{32}$P treatment;
More than 100 $\mu$Ci for meiotic stage in rice
100 $\mu$Ci for pro-embryonic stage in rice
20~50 μCi for meiotic stage in wheat
Regarding ³²P treatment;
150~200 μCi for meiotic stage in rice
50~80 μCi for meiotic stage in wheat

Developmental stage at treatment-effect relationship.

In the treatments by ³²P or ³⁵S at a certain stage during reproductive growth, there was general tendency that the later treatment took place, the higher fertility was obtained. Concerning this tendency, it has been found that almost complete fertility was obtained in the ³⁵S treatment at pro-embryonic stage in rice even for rather high dose, but germinability or viability of the resulted seeds was very low.

On the mutation, Kepper et al. (1961) has assumed that high yield of mutation might be expected in the treatment during from late period of gametogenesis to pro-embryonic stage after the experiment of ³²P treatment in barley.

The result of my experiment about ³⁵S treatment by injection method at three stages of reproductive growth in rice was shown in Table 1. According to this
data, the treatment at just prior to meiosis gave the highest mutation rate. A consideration can be made with this reason that only the treatment at just prior to meiosis actually covers internal irradiation in meiotic process, but treatment at meiosis does not cover this process, because some lagging time is observed between the time of injection and the beginning of biological action.

If above mentioned consideration is acceptable, it will be able to say that meiosis is very sensitive stage for induction of mutation.

3. A Comparison between RI Absorption and X or 7 Irradiation in their Effects on Induction of Mutation

Since the pattern of action of RI absorption differs from that of external
radiation, it is difficult to compare the biological effects between them in a strict sense.

But it must be very interesting for us to try to compare the effects on some final results from the practical standpoint.

Cytogenetical and mutational effects of RI absorption have been compared with those of external radiations by several workers.

The conclusions sometimes vary by workers with difference of their materials used and items of observation, but many of them found that RI absorption gave higher mutation frequencies than external radiations in the seed treatment and also there were some differences in mutation spectra between two methods (Shesbeski et al., 1954; Mazima et al., 1957; Pal et al., 1958; Shastry et al., 1961 and Kawai, 1963).

A simple comparison on induction of chlorophyll mutation in rice between RI absorption including the treatment for both seeds and growing plants and X or 7 irradiation for seeds will be presented next.

The same rice variety Norin No. 29 was used as a material in all of the treatments employed in this comparison and the observation methods were almost the same.

Mutation frequency.

According to the data in Table 2, possible maximum mutation frequency in the seed treatment by X or 7 irradiation is thought to be around 7 per cent in

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth in R1 (% of controls)</th>
<th>No. of mut. per 100 R1 panicles</th>
<th>Reporter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seedling height</td>
<td>Survival rate</td>
<td>Seed fertility</td>
</tr>
<tr>
<td>X-ray, 20kR</td>
<td>-</td>
<td>-</td>
<td>63</td>
</tr>
<tr>
<td>74 kR</td>
<td>74.3</td>
<td>67.3</td>
<td>42.7</td>
</tr>
<tr>
<td>70 kR</td>
<td>45.8</td>
<td>48.3</td>
<td>24.1</td>
</tr>
<tr>
<td>20 kR</td>
<td>-</td>
<td>-</td>
<td>71.3</td>
</tr>
<tr>
<td>15 kR (Pre-soaked)</td>
<td>-</td>
<td>-</td>
<td>54.9</td>
</tr>
<tr>
<td>7-ray, 30kR</td>
<td>-</td>
<td>-</td>
<td>34.0</td>
</tr>
<tr>
<td>30 kR</td>
<td>-</td>
<td>50</td>
<td>41.0</td>
</tr>
<tr>
<td>2µCi/seed</td>
<td>93</td>
<td>82</td>
<td>82</td>
</tr>
<tr>
<td>2µCi/seed (Phosphorus deficient seed)</td>
<td>63</td>
<td>59</td>
<td>78</td>
</tr>
<tr>
<td>100µCi/stalk (At meiosis)</td>
<td>89.0</td>
<td>89.5</td>
<td>91.0</td>
</tr>
<tr>
<td>300µCi/stalk (At meiosis)</td>
<td>79.3</td>
<td>78.6</td>
<td>74.5</td>
</tr>
</tbody>
</table>

Treatment with X- or 7-ray means acute irradiations to seeds.
R₁ panicle base with dose of about 30 kR, considering the physiological damages and seed fertilities.

On the other hand, the seed treatment by ³²P absorption, even if omitting the special case of the treatment for phosphorus deficient seeds, will easily be able to keep the frequency more than 8 per cent.

Mutation frequencies obtained from the treatment by ³²P and ³⁵S at meiotic stage are not so high. However, at present time the possibility of RI absorption for growing plants is almost unknown. For example, the decrease of growth and seed fertility in R₁ were very slight in the ³²P treatment at meiotic stage which gave 5.33 per cent of mutation frequency. Therefore, in this case the higher mutation frequency may be expected by applying the higher dose.

*Mutation spectrum.*

Data concerning on this point is shown in right hand of Fig. 4. Some differences are clear among these treatments, but general rule may not be drawn to distinguish the effect of RI absorption from that of X irradiation.

Differences on the frequency of albina and viridis mutation are found between ³²P treatment for seed and meiotic stage. As it were, the difference which is due to treated stage with ³²P absorption is more distinct.

Furthermore, large decrease of viridis mutations in ³⁵S treatment at pre-meiotic stage is attractive. And it is very interesting to consider the fact that the same tendency was found in ³⁵S treatment of barley seeds. But it can not be decided by these limited cases whether it is a characteristic of ³⁵S or not.

*Segregating fashion of mutation.*

Frequency distributions of segregation ratio of R₁ panicles were obtained in each treatment from the observation in R₂ plants and these were shown in the left side of Fig. 4.

In all treatments, except the ³²P treatment at meiosis, the modes of segregation ratio are 20–25 per cent and mean values and shapes of curve are also not so different among treatments.

Only the ³²P treatment at meiosis has no mode of segregation ratio, therefore, its mean value becomes low.

This indicates that the panicles derived from this treatment contain many individuals with smaller size of mutation sector. These facts suggest that, in this treatment, more mutations might have occurred at later stage of development than in other treatments.

While, the sizes of mutation sector within R₁ plant (ratio of number of panicles with mutation to number of panicles in each R₁ plant with mutation) have also been compared among four kinds of treatments i.e., ³²P at meiosis, ³⁵S at just
Segregation ratio in R₁ panicle (%)  
Fig. 4. Comparison between RI absorption and X irradiation about mutation spectrum and segregation ratio of chlorophyll mutation induced in rice variety, Norin No. 29.  
M : Mean value

prior to meiosis and X irradiation for both dormant and pre-soaked seeds. In 67~77 per cent of R₁ plants with mutation, sector sizes were less than 0.4 throughout all treatments. And no principal difference was found between RI absorption and X irradiation.

References

3. KAWAI, T. (1963) Mutation in rice induced by radiation and their significance in rice
RI 内部吸収による植物の突然変異誘発効果

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(筑城大学農学部)

(1) 内部吸収の生理的、遺伝的効果は、同じ材料、同じ RI を用いているにもかかわらず報告者によっていちじるしく異なる場合がある。この理由は主として、処理条件のちがいにより吸収量が異なるためと思われる。

種子処理では、RI の吸収は主として発根後数日によって行なわれる。したがって一般に乾燥種子から処理するよりも、あらかじめ催芽した種子を用いた方が、処理時間を短縮できる点で、また処理種子の腐敗や窒息死を防ぐ意味で、はるかに合理的であろう。トマト の 32P 处理実験では、発根後数日で十分な RI の取り込みが認められたが、一般に発根後 1 週間以上処理すればほとんど 100% 近い吸収率を確保できるものと思われる。

処理時の RI 濃度も吸収効率に影響するが、トマト、イネおよびコムギでの実験結果によれば、種子容積の 5～10倍が最も合理的と認められた。また圃場や砂の上での、種子が直接 RI 溶液中に浸しかりにして処理することにより、幼植物の生長は著しいもの。しかしこの場合吸収率は低下する。トマトの 32P 处理実験では、直接浸漬にくらべて圃場の場合 70%，砂では30%となった。

吸収率の個体間変動は発芽の遅速に基づくが、これがあまりいちじるしいと処理効率を悪くする。そこで、とくに発芽不揃いの作物の種子処理に当たっては、あらかじめ催芽した種子から発芽段階の等しいものを選別して供試する必要がある。

特殊な処理方法としてイネでリン欠種子の 32P 处理を試みた結果、リン含量が通常の50%程度の種子を用いると突然変異率は通常種子の約 2 倍になることが認められた。

(2) イネ、コムギおよびオオムギを用いて生殖生長期における生体処理の効果をみた。

処理方法は水耕によるのが処理の精密さの点からは最もすくれているが、簡便さの点から節間注入処理が実際的であった。しかし、32P は 32P にくらべて体内移行性が乏しいので、注入部位によって特定の組織や器官での集積量が異なる。
生体処理の結果体内に器官に集積する RI 量は、通常の Dose の範囲内では、Dose の強さにほぼ比例し、突然変異率もかなり、これを平行的であった。

生育段階と効果との関係は、現在十分なデータがない。イネの 35S 処理では、その生殖生長期のうち減数分裂期が突然変異の誘起に対して敏感なように思わわれた。

(3) RI 内部吸収と X (7) 線によって、イネ農科29号に誘発された葉緑素突然変異と比較してみると、RI 内部吸収の突然変異誘発効果は少なくとも X 線や γ 線のそれに劣らない。

R1 の生存率や種子稔性等を合わせ考えると、生体処理による場合を含めて、変異率を現在の水準より高めうる可能性がある。

しかし、この比較ではそのスペクトルが、とくに外部照射と異なっている根拠はみだせなかった。スペクトルのちがいは、むしろ同じ 32P 処理における種子処理と減数分裂期処理との間に、また 35S 処理とその他の処理との間に認められた。

さらに突然変異の R2 における出現の様相を R1 種内分離比および R1 個体内セクターサイズによって比較してみたが、RI 内部吸収と X (7) 線照射との間でとくに差異を認めることはできなかった。

質 疑 応 答

広部 問題にした突然変異はすべて葉緑素突然変異ですか。
小野沢 そうですね。
広部 葉緑素突然変異は、albino, viridis 等を一括したものですか。
小野沢 そうですね。
広部 葉緑素突然変異のスペクトルについて内部照射と外部照射の間に質的な差がありましたか。
小野沢 現在のところ、はっきりした差が出ておりません。
広部 形態的突然変異においても、内部照射と外部照射の間に、質的な差異が生じなかったのですか。
小野沢 その点について多少の差があるとの報告もありますが、私は実験をしていないので、今はっきりと述べることはできません。

私は、内部照射の場合には、むしろ用いられる元素——たとえば 3H, 14C, 32P, 35S——の種類による突然変異の質的な差の方が大きいものと考えています。
先ほど申し忘れましたが、35S の葉緑素突然変異のスペクトルは 32P と少し違ってい

松村 突然変異のスペクトルの図で、virido-albino 等はどの分類に入れましたか。
小野沢 「その他」に入れてあります。
INDUCTION OF MUTANTS IN SWEET POTATO
BY ABSORPTION OF $^{32}$P

Kenkichi Sakai

Kyushu Agricultural Experiment Station, M. A. F., Kumamoto-ken, Japan

Concerning the induction of artificial mutation, Meguro (1942) has reported
the breeding of tetraploid sweet potato; Poole (1959) and Helnandez et al. (1964)
has reported the induction of mutants by gamma-ray irradiation; and Majima et al.
(1959) and Marumine et al. (1962) have reported the induction of mutants by
X-ray. On the other hand, Akita et al. (1960) has been trying to breed the
practical variety from the true seeds which were exposed to gamma-rays.

The present experiment was conducted in order to breed parental lines with
high starch content, which is the most important objective in the sweet potato
breeding in Japan. Experiment consisted of following three trials:

1) Observation of mutants by absorption of $^{32}$P for several characters and
selection of mutants with high starch content.

2) Accumulation of variations for high starch content and selection of mutants
with high starch content by every second or third year absorption of $^{32}$P.

3) Observation of mutants by absorption of $^{32}$P for several characters of
extremely high starch content individuals and selection of mutants with extremely
high starch content.

1. Method of Treatments

Treatment were carried out on the commercial varieties, and the methods of
treatment were three as follows:

1) Method of absorption by cutting

Two kinds of cutting were used, the one is the cutting with developed roots
which were cut one week before treatment; and the other is the cutting which
were cut on the day of treatment. These cuttings were put into the beakers, in
which various doses of $^{32}$P were prepared to be absorbed by the cuttings.

2) Method of spread on the leaves of cutting

Various doses of $^{32}$P were spread on the leaves of cutting which were mentioned
above.

3) Method of injection into tuber

Various doses of $^{32}$P were injected into the hole about two cubic centimeter on

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M.A.F., Yotsukaïdō, Inba, Chiba Pref., Japan.
the seed tuber, and the sprouts from the treated tuber were used.

The method of absorption by cutting was better than that of spread on the leaves of cutting because of simplicity of treatment, and in the case of absorption it is profitable to use the cutting with developed roots. The rate of absorption was about 60 per cent calculated in terms of treated stage in each case, but in the case of spread the whole amount was not always absorbed.

The injection into tuber seems good method, because the process is simple and can deal with many cuttings at a time. In this case, there were the varietal differences on the rate of absorption of all cuttings per one tuber; 'Kyushu No. 38' absorbed about 30 % and 'Okinawa No. 100' absorbed about 60 %, respectively. Then the rate of absorption by long or the top cuttings were much higher than that of short or the tail cuttings.

2. Propagation of Mutant and Method of Selection

Several rudimentary organs of roots exist in the base of a leafstalk of cuttings, and they develop to become tubers. Therefore it may be considered that the rudimentary organs receive action of $^{32}$P which was absorbed by the cutting. Rudimentary organs consists of many cells which is developed from one original cell, so it may be supposed that the mutants of treated generation ($R_1$) were not uniform but chimeric condition. From this point of view, the method of injecting into tuber is in different condition. In this case, the rudimentary organs of sprout exist in the tuber, but the rudimentary organs of tuber do not exist in the rudimentary organs of sprout. Therefore, the tubers of $R_1$ were not affected immediately by $^{32}$P but mediatley through the cuttings. So, it may be supposed that the tubers of $R_1$ were uniform mutants. In any case, it is considered that the variations occurred in the tubers of $R_1$ and that the variations were in uniform or chimeric condition.

Although chimera is not a desirable from in the vegetatively crops propagated, it seems that chimera happened, in fact so it is a question to find the mutants and how to propagate itself. In this trials, the materials were handled as follows in order to find a solution of the above problem without the regards to the method of absorption by the cutting or injection into the tuber.

1) The second year clones cultivated as tuber were one line from each $R_1$ tuber. One plant was grown from one sprout which sprouted from a tuber of $R_1$ and 12-15 sprouts were planted, so the condition of variation was observed for the second year clones. However, it is difficult to catch the small genetic variations of quantitative characters which are determined by the effects of polygenes.

In order to catch the small genetic variations of quantitative characters, it is
essential to secure, in some degree, the number of individuals to control the environmental variations.

2) In the clones on and after three years from R₁ tubers, clones from one plant of the second year clones were cultivated as one line, moreover clones from one tuber from these lines were subdivided and propagated as one sub-line.

3) The final objective of these trials is to induce mutants of yield and starch content (this is estimated by dry-matter percentage or extraction rate of starch) which are considered to be determined by the effects of polygenes, so it is estimated the extraction rate of starch by dry-matter percentage presently and selected the high dry-matter percentage mutants and the low ones. Selections were continued until 3 or 4 years clones on and after the second year clones in which mutants were observed to have been occurred, and we threw away clones except the highest or lowest ones.

3. Power of Dose and Number of Times of Treatments

Quantity of treatment was 20µCi per one cutting as prescribed in case of the method of absorption by cutting (quantity of absorption was 15.2 µCi per one cutting as calculated in terms of the time of treatments), and from 300 µCi to 2400 µCi per one tuber in case of the method of injection into tuber (quantity of absorption was from 5 to 25 µCi per 10 grams cutting of tuber as calculated in terms of the time of treatments). Therefore, the method of absorption by cutting is better than that of injection into tuber in the case of absorbing large dose at a time. As for the number of treatments, in trial 1) only one time treatment was conducted in 1960, when 20 µCi of ³²P were absorbed per one cutting, and after that the selections of individuals and lines were continued. In trial 2) of one treatment for two years, 1st time treatment was conducted in 1960, when 20 µCi of ³²P were absorbed per one cutting and the selection for the higher starch content was made individually next year, then in 1962 treatment was conducted, when 300 µCi of ³²P was injected into one tuber as the 2nd time treatment, and the selection for the higher starch content was also made individually next year. Then in 1964, the 3rd time treatment was conducted, when 300 µCi of ³²P was injected again into one tuber that was selected in 1963, and the same selection was done. On the other hand, in the case of one time treatment for three years, the 1st treatment was conducted in 1960, when 20 µCi of ³²P per one cutting were given and the selections of individuals and lines were continued next two years. Then in 1963, the 2nd treatment was conducted, when 800 µCi of ³²P was injected into one tuber and the selections were continued next two years as mentioned above. In trial 3) several varieties were used and treated by the method of in-
jection into tuber with the dose of 150, 300, 600, 900, 1200 and 2400 μCi of $^{32}$P per one tuber, respectively, and the selections were continued on and after treatments.

4. Variations of Top

1) Effects for sprouting habit in the nursery

Sprouting habit of the $R_1$ tubers was observed in this case. The trial was conducted in which various doses of $^{32}$P were injected into the tubers of ‘Kyushu No. 34’ in 1962. The result is summarized in Table 1. As seen in Table 1, the

<table>
<thead>
<tr>
<th>Dose per one tuber (μCi)</th>
<th>Length of sprouts (cm)</th>
<th>No. of nodes</th>
<th>No. of sprouts</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>23.0</td>
<td>10.8</td>
<td>26.2</td>
</tr>
<tr>
<td>300</td>
<td>21.2</td>
<td>11.2</td>
<td>29.5</td>
</tr>
<tr>
<td>600</td>
<td>19.5</td>
<td>9.7</td>
<td>27.0</td>
</tr>
<tr>
<td>900</td>
<td>18.5</td>
<td>12.5</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Note: *Does per one tuber was prescribed.

length of sprout had a tendency to become shorter according as the increase in dose, but there were no differences on the number of nodes and sprouts. The results of trials which were conducted by the above-mentioned method using ‘Satsuma-aka’ and ‘Kyukei No. 15-2120’ in 1965 are given in Table 2. The figures in Table 2 show the total length and the total weight of cuttings of planting age.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Total length of cutting (cm)</th>
<th>Dose per one tuber (μCi)</th>
<th>0</th>
<th>370</th>
<th>740</th>
<th>1480</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Satsuma-aka</td>
<td></td>
<td>120</td>
<td>220</td>
<td>162</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Kyukei 15-2120</td>
<td></td>
<td>270</td>
<td>140</td>
<td>227</td>
<td>485</td>
</tr>
<tr>
<td></td>
<td>Total weight of cutting (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Satsuma-aka</td>
<td></td>
<td>144</td>
<td>153</td>
<td>124</td>
<td>183</td>
</tr>
<tr>
<td></td>
<td>Kyukei 15-2120</td>
<td></td>
<td>199</td>
<td>91</td>
<td>135</td>
<td>311</td>
</tr>
</tbody>
</table>

Note: *This shows the practical dose at the time of treatment. In this case, the prescription of dose was 0, 800, 1200, 1600 μCi respectively.

This figure shows that, there were no effects on the two characters according to the increase in dose. Although the statement of the following things can not be
made positively because the number of observations was so small, judging from
these facts above mentioned, it is considered that there were no effects on the
sprouting habit in the nursery as far as the degree of dose is concerned in this
trials. If the effects on sprouting habit are to be expected to appear on the surface
according as the increase in dose, then it will be necessary to use larger dose.

2) Effects for total length of stem, stem colour and stem diameter in field

The results of observations of total length of stem per one plant on second

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Dose per tuber</th>
<th>Quantity of absorption for estimated per one cutting of 10 grams</th>
<th>Total length of stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cm</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S 106-464</td>
<td>690</td>
<td>5.3</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>1030</td>
<td>5.9</td>
<td>149</td>
</tr>
<tr>
<td></td>
<td>1380</td>
<td>7.0</td>
<td>149</td>
</tr>
</tbody>
</table>

Kyushu No. 38

|                |                |                                                                |       |          |                              |
|                |                |                                                                |       |          |                              |
| 158           | 1.0            | 103                                                            | 1930  | 705      | 43                           |
| 316           | 2.5            | 91                                                             | 1997  | 1801     | 49                           |
| 632           | 3.7            | 83                                                             | 1807  | 1036     | 51                           |
| 1264          | 7.7            |                                                                |       |          |                              |

Okinawa No. 100

|                |                |                                                                |       |          |                              |
|                |                |                                                                |       |          |                              |
| 158           | 2.4            | 93                                                             | 2148  | 852      | 50                           |
| 316           | 4.5            | 87                                                             | 1863  | 852      | 49                           |
| 632           | 6.1            |                                                                |       |          |                              |
| 1264          | 10.6           |                                                                |       |          |                              |

Note: *This shows the practical dose at the time of treatment. In this case,
the prescription of dose was 800, 1200, 1600μCi at S 106-464 and 0,
300, 600, 1200, 2400 μCi at others respectively.

year clones are given in Table 3. The grass-types of three varieties used in this
trials were different in character that is 'S 106-464' is short with many ramification
'Kyushu No. 38' is medium with many ramification and 'Okinawa No. 100' is long
with few ramification. Judging from Table 3, there was no difference in 'S104-
464', short and many ramification grass-type, but medium or long type of 'Kyushu
No. 38' and 'Okinawa No. 100' had a tendency to become shorter according as
the increase in dose. On the other hand, the mutants of slender stem and the
longer stems had been seen in 'Okinawa No. 100' as shown in Table 4. From
this point of view, it seems that the variations of stem length show tendencies to take shortening or lengthening directions.

<table>
<thead>
<tr>
<th>Dose per one tuber</th>
<th>Stem colour</th>
<th>Stem diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µCi</td>
<td>Normal</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>158</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>316</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>632</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>1264</td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Three times treatment</th>
<th>Stem colour</th>
<th>Stem diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µCi</td>
<td>Normal</td>
</tr>
<tr>
<td>5-1</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>19-2</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>36-1</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td>37-3</td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

Note: * Same as Table 3.
** In 1960, 32P at rate of 20 µCi per one cutting, and in 1962, 1964 32P at rates of 300 µCi per one tuber respectively.

The results of observations of stem colour and stem diameter on the second year clones are shown in Table 4. The usual stem colour of 'Okinawa No. 100' is green with a tinge of purple, however, the green stem with no tinge of purple appeared in the most high dose of 1264µCi of 32P. Then the stem diameter of 'Okinawa No. 100' is usually large, but the small stem appeared one in each dose over 316µCi of 32P respectively.

Four groups of lines of 'Okinawa No. 100', the materials of trial 2), were treated three times in 1960, 1962 and 1964. These groups were selected by high or low dry-matter percentage, so the comparison cannot be made strictly, but many mutants of various stem colours and diameters appeared in 37-3 groups and appeared fairly well in other groups. As no examples has been reported about the frequency of the spontaneous mutation, we cannot say strictly, but it may safely be said that the frequency of mutations in these trials are higher than that of spontaneous ones. From the above-mentioned results, it is considered that the mutants of both characters were increased according to the increase of the treatment time.

5. Variations of Root

1) Type of tuber and skin colour
The abnormality of tuber was found occasionally, but that was not hereditary.
Skin colour did not vary as we had expected except only one mutant of dark red which was found in 'Kyushu No. 38'.

The frequency of the mutations for skin colour was described (Fujise, 1965) at symposium of last year. The number of the mutations for skin colour varied from zero per cent of minimum to 7.5 per cent of maximum and 1.9 per cent on the average among about twenty varieties according to the report above-mentioned. Therefore, it seemed that the frequency of the mutations of skin colour on this trial was not higher than that of spontaneous ones.

2) Yield of tuber and dry-matter percentage or extraction rate of starch

It is important but very difficult to find and breed the mutant tubers which have higher dry-matter percentage or extraction rate of starch. Especially, the environmental variations of the yield of tubers are very large in sweet potato, so the variations of dry-matter percentage which have comparative small environmental variations was chosen as the object of observation.

<table>
<thead>
<tr>
<th>Table 5. Materials in 1965</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okinawa No. 100</td>
</tr>
<tr>
<td>Origin</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>One time treatment and no selection</td>
</tr>
<tr>
<td>Three times treatment and selection for high and low dry-matter percentage at each times</td>
</tr>
<tr>
<td>High (Okie 100-5-1)</td>
</tr>
<tr>
<td>High (Okie 100-19-2)</td>
</tr>
<tr>
<td>Low (Okie 100-36-1)</td>
</tr>
<tr>
<td>Low (Okie 100-37-3)</td>
</tr>
<tr>
<td>Comparison</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Standard (no treatment)</td>
</tr>
</tbody>
</table>

Note: * Origin of comparison as follows:
PM60H101 | In 1960, $^{32}$P at rates of 20 μCi per one cutting, and selections continued from 1961 to 1964 for high (H) or low (L) dry-matter percentage.
PM60H381
PM60L102
PM60L382
PM60H103
PM60H383
PM60L104
PM60L384

No treatment, and selections continued from 1961 to 1964 for high (H) or low (L) dry-matter percentage.
Dry-matter percentage were observed individually on the materials of Table 5 which could be compared with the lines of the second year clones in 1965. Ten plants were used in one line. Now the comparison of the variance of the dry-matter percentage with the lines of different origin is given in Table 6. According to Table 6, it seemed that the number of lines were different, but the size of

Table 6. Comparison of variance for lines of different origin

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Standard</th>
<th>Comparison</th>
<th>1st time treatment</th>
<th>Three times treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okinawa No. 100</td>
<td>No. of line</td>
<td>18</td>
<td>72</td>
<td>78</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>28.4</td>
<td>28.7</td>
<td>29.2</td>
</tr>
<tr>
<td>Variance</td>
<td></td>
<td>1.00</td>
<td>3.40</td>
<td>1.77</td>
</tr>
<tr>
<td>Kyushu No. 38</td>
<td>Mean</td>
<td>36.0</td>
<td>36.0</td>
<td>36.4</td>
</tr>
<tr>
<td>Variance</td>
<td></td>
<td>7.37</td>
<td>2.88</td>
<td>4.04</td>
</tr>
</tbody>
</table>

variance were in following order, three times treatment > comparison > one time treatment > standard in 'Okinawa No. 100'. In 'Kyushu No. 38', the comparison cannot strictly be made because the standard shows abnormal growth, but the variance had a tendency to become larger according as the increase in times of treatments and selections. From this point of view, treatment and selection have the effect of enlarging the variance of dry-matter percentage, and moreover, it is more effective to repeat treatments.

As it is stated previously the mutants of the stem colour and stem diameter were found much in three times treatment. Now, it is considered that repeated treatment is the good method to induce the mutants for dry-matter percentage. In this trial the increase in dose were not found in the dry-matter percentage.

References


부기 야마토의 인플루언스 변화의 유발

坂 井 健 吉

(九州農業試験場)

この実験は、現在わが国のサツマイモの育種で最も重要な目標となっている、高でん粉多収性品種を育成するために、高でん粉母本を育成する目的で試みられた。

材料と方法

部酸によるサツマイモの個体変異の調査ならびに高でん粉個体の選抜を目的として、まず1960年に、沖縄100号と九州38号を供試し、それぞれの切苗に1本当たり約20μCiを吸収させ、直に植付けて照射1年目（R1）の塊根を養成した。同時に苗の葉に部酸を塗布する方法も試みた。R1の塊根はそのまま保存し、翌年の塊根から出た苗を1芽1系統（1株）として、栄養系2年目を養成し、系統ごとに塊根1個により、切干歩合（でん粉含糖を推定する形質）を調査するとともに、翌年の種いもを保存した。栄養系3年目の2年目の系統（1株）を、さらに系統内で塊根1個を1系統として分系増殖し、切干歩合の高低により選抜を加え、高低両端の系統を保存した。栄養系4年目以降は同様の方法で、切干歩合の両極端の系統のみ選抜保存し、5年目まで養成した。

次に収量やでん粉含量のような、ポリジーンに支配されると思われる量的形質は、変異の累積効果がみられるのではないかと考えられるので、60年苗処理を行ない、61年切干歩合の高低により選抜した他の材料につき、63年種いも1個当たり300μCiの処理を行ない、さらに同様の方法で、61年選抜したものに65年再び300μCiを処理した。処理の方法は、種いもに約2cmの穴を穿ち、部酸の稀釀所定液を注入し、それより萌芽した苗を植付けてR2を作るものである。部の陽日から萌芽した別の材料につき、3年1回、1個当たり800μCiを処理した実験も試みた。この他、61年より極高でん粉品種、九州34号など数品種を用い、主として種いも注入法で、1個当たり150μCiから2,400μCiを処理し、上述の方法で変異の状態を調査するとともに、極高切干個体を選抜した。

地上部の変異

部酸を注入した種いもの苗床における萌芽性は、九州34号では、線量の増加とともに萌芽長が短くなったが、萌芽数数や萌芽数には差がみられなかった。またサツマアカリや九州12-2120では、線量の増加による萌芽性の差は明瞭ではなかった。処理した苗の本体にあたる茎長は、草型が短苗のサツマアカリでは、処理平均値間で差がみられなかったが、中蔓や長蔓の九州38号や沖縄100号では、平均値間に差が見られ、線量の増加とともに短苗化の傾向がみられた。茎色や茎径の変異体は、3回処理3回選抜の沖縄100号にかなり大きな頻度で出現した。茎径の細くなったものは、長蔓化し易いが、この結果、茎は長短いずれ
の方向にも、かなりの頻度で変異することが明らかになった。

地下部の変異

いもの形では、沖縄100号に奇型がみられたが、遺伝的なものではなかった。皮色は期待した程に変わらず、僅かに赤紅色の九州38号に1個淡赤色のものがみられたのみであった。皮色の変異は、自然条件でも、かなりの頻度で起こることが報告されている。この実験では、数品種を用い、いろいろの処理を行なったにもかかわらず、きわめて稀であったが、これについてはさらに高い順序で検討する必要があるよう。

塊根の収量は、環境変異が大きく、確実な変異体を把握する段階には至らなかった。切干分合では、栄養系2年目として、系統比較試験ができる由来の異なった材料につき、系統（株）平均値の分散を、処理分かと処理間の大きさによって比較した。その結果、切干分合の分散の大きさは、沖縄100号では標準（無処理無選抜）＜初回処理（1回処理無選抜）＜比較（1回処理5回選抜）＜3回処理（3回処理3回選抜）の順となり、九州38号でも、同様の傾向がうかがえた。しかし割量の増加による、分散の大きさの差異はみられなかった。

以上の結果、この実験から32Pの吸収によるサツマイモの主根変異の透発に関しては、処理・選抜の繰返しが最も効果的であると結論される。

質疑応答

松村 種子繁殖の植物で使われているR₁, R₂等の呼称に代わるべきところの、栄養繁殖の植物の符号について何か考えていますか。

坂井 別に私の考えはありません。

松村 私は根抽氏がチューリップで使っているようなVの符号を用いて、たとえば、R₂ (R'₃) をV₂₃とするのも好例ではないかと思っております。

明峰 選抜試験において、無処理区にも選抜の効果があるようになっているのは、どんな理由によるのですか。

坂井 最初の試験では処理の効果はなくて選抜の効果だけがあったと考えていますが、ここまでもってきても試験では連続処理の効果が多少でているものと思っています。

広部 個体選抜をする場合、多数の個体を選抜しないということは、結局、個体選抜をしてということになると考えられます。環境とか年次による変動を考慮して、多数の個体を選抜し集団を大きくすべきではないでしょうか。
総合討論

河合 本日の三氏の講演の要点を述べますと、池永氏は、細胞あるいは分子レベルで内部照射の作用を比較し、内部照射では原子の崩壊による放射線の効果があるが、原子の崩壊の効果が微生物の場合のように高等動物でも見られるかどうかを問題にしました。カイコでの実験の結果は否定的で、高等動物では崩壊による効果が放射線による効果に比較して少ないために、実際には観察することができないのであろうとしています。

小野沢氏はイネを使って、$^{32}$Pによる種子処理、$^{32}$Pと$^{35}$Sによる生体処理およびX線、ガンマ線の種子処理による障害、突然変異率、セクターの大きさ、突然変異のスペクトラムを比較し、突然変異の出現に関してはこれらの処理の間にあまり大きい差がなかったとしています。

坂井氏は、栄養繁殖植物であるサツマイモにおける$^{32}$P処理による量的形質特に澱粉含有量および収量の変異の誘発と選抜の実験の結果を述べ、この場合に内部照射によるキメラの発生を考えし、処理2年目栄養系での個体単位の選抜と処理3年目栄養系での群としての選抜を行なっております。その結果、処理と選抜のくりかえしにより分散が増加することが認められ、澱粉含有量の突然変異が誘発されたと推定しております。また澱粉含量について高い方向、低い方向への選抜を行なった結果についての報告がありました。

では、まず分子あるいは細胞レベルでの問題から討論を始めたいと思います。

代谷 $^{32}$Pのベーター線の最大距離は相当大きいし、さらにRNAなどへの影響も考えられるので、池永氏の実験結果で、原子の崩壊による効果は見掛け上は観察されなかったと考えてもよいのでしょうか。

池永 大体そのように理解してもよいでしょう。しかしあっての推量ですが、高等動物の染色体は$^{32}$Pなどの原子崩壊による物理的ショックでDNAの一部がブランクになっても、なおかつ生物活性を保持できるような構造、あるいはDNAが次のreplicationまでに酵素などによって修復されるような構造を持っていることなどを考えられないことはないでしょう。一方、大腸菌では、DNAがむき出しの状態になっているが蛋白質がまわりにまといついているから、DNAのバックボーンの切断に対して感受性が高いと考えられます。

河合 池永氏は、高等動物植物ではDNA strandのまわりを核蛋白がとりまいているので、$^{32}$Pが$^{35}$Sに変わった時に、微生物の場合とは異なった反応を示す可能性があるとの意見でした。核蛋白にとりこまれる$^{35}$Sの作用について、何かデータがありますか。

池永 私の知る限り、高等動物植物の遺伝学的な面での研究データはないです。微生物では、DNAの切れことが本質的な効果であるから、それから当然予測されるように、$^{35}$Sでラベルした大腸菌はほとんど死ぬことがないというデータがあります。

（Strauss, 1958 Stent and Fuerst, 1960）。

松尾 坂井氏がツツマイモの突然変異育種を始めるにあたって、マクロな突然変異は、部
池永 私自身は、突然変異誘発源の種類の違いよりも、むしろ総線量の方が効いてくるのではないかと考えています。たとえば X 線でも、低線量では激しい障害はあらわれなくても、相対的な意味でポリジーン的な突然変異がおこりやすいのではないかと思っています。一般に障害の程度は LET の面倒と考えられ、³²P の LET は 200eV/μe の X 線に近いので、障害についてはほぼ同じ程度の作用を与えるものと思われます。私にとっては、育種的に見れば、むしろ化学物質を使うべきだと思います。

松村 低線量は弱線量であるべきではないでしょうか。

池永 両方を含めて考えています。

山口 LET の計算の場合同に、ベータ崩壊による反跳原子核の効果をどのように評価すべきでしょうか。

池永 この種の仕事で、従来は LET の計算には反跳原子核の効果を無視していたのですが、これからは、反跳原子核の効果を考え入れるべきでしょう。実験的には反跳原子核の効果を分離して評価することは非常に困難なので、反跳原子核および transmutation の効果という具合にひっくるめて考えているのです。

山口 ベータ崩壊では原子価の変化ベータ線の放出が主なる物理的変化でありますが、その他の反跳原子核によるイオン化も少しあるため、LET はもう少し高くなるような気がします。

池永 実際そうでしょう。Oak Ridge でのパクテリオファージの最新のデータによりますと（Steinberg and Going, 未発表）、ベータ線のエネルギーが低い³²P を使った場合には、ファージの死に方が³²P の場合の約 1/5 になっています。ファージの場合にはベータ線の寄与は計算上はでこないので、恐らく原子核の変換よりも反跳原子核の方が本質的に効いているものと考えています。

山崎 微生物と高等生物の比較になりますが、transmutation を考える場合に、染色体の異常が起こるものと想像されます。したがって、染色体の観察が transmutation の問題を研究する時の一つの手だてになるかどうかを教えていただきたい。

もう一つは、微生物の DNA は“はだか”であって作用されやすく、一方高等生物のそれは蛋白質に囲まれているため影響を受けにくいということでしたが、これは³²P のとりこみ方について、両者の間に差を生じさせるものと考えられます。この点についてのご教示もいただきたい。

池永 染色体異常についてはですが、カイコの卵の突然変異は恐らくそれに起因しているものと考えています。³²P 処理とガンマー線照射の間で差が見られなかったことは、多分、染色体異常についても、両者の間に差がなかったからだと想像しています。ポジティブな transmutation の効果を明らかにするための手だてとして、染色体異常を観察するのはあまり有効なことは考えられません。

第２の問題ですが、微生物とカイコ、特にその精巣への³²P のとり込みについては、本
質的な差はないものと思います。大腸菌の実験で普通に使われている量（大体、10mCi／mg）の³²Pをカイコに注射しますと、恐らくカイコは完全に死んでしまうでしょう。これは微生物とは違って、カイコでは一つの細胞群が隣接する細胞からのペルタ線の影響を受けけるからです。その意味で実験はできませんが、とりこめらせて、両者とも同じ程度に³²Pをとり込むものと考えています。

河合 次に個体レベルの問題の議論に移りたいと思います。

種子処理では、外部照射と内部照射の間に突然変異率、セクターの大きさ、突然変異のスペクトラムについて大きな差がなかったという報告であったと思います。内部照射は一種の緩照射でもあり、外部緩照射と比較しての御意見をうかがいたい。

田中 先ほどの細胞レベルの議論で、総線量と線量率の関係が問題になりましたが、私達がおこなっている生育中のガンマ線照射実験では、大体、障害は線量率に依存し、一方突然変異率には線量が及ぼすという結果になっています。イネの場合、低線量率で長期間照射すれば、総線量に比例して突然変異率は高まっていくのが普通です。河合氏の種子処理のデータでも、突然変異率はX線外部照射、³²Pの内部照射、³⁵Sの内部照射の順に高くなっています。

河合 セクターの大きさについての御意見は？

田中 山口氏が発表しているように、種子照射では一般的に高線量の照射ではセクターが大きくなります。また当然のことですが、変異が誘発された時の作物の生育時期によって、セクターの大きさはかわってくる。

また、diplonticあるいはhaplontic selectionも含らくでしょう。しかし、種子繁殖作物では栄養繁殖のものに比べて、セクターのこれらselectionの問題をそれほど神経質と考えなくてもよいでしょう。もっともセクターの大きさは育種年齢を考える時には当然問題になりましょう。

河合 ³²Pの内部照射についてのその他の実験結果を紹介していただきたい。

小林 主として数分裂期前の注入によって、突然変異率の高まることは確かでしょう。特に半減期の短いものについては、そのようにすることが方法論的に正しいものと思います。

河合 最後に、栄養繁殖植物における量的形質の突然変異の選抜の問題に入りたいと思います。

蓬原 量的形質の場合に、連続照射は非常に重要だと考えています。

まずマイモの実験で、3回照射によって分散が大きくなったとのことでしたが、平均値はどうでしたか？また変異の偏りはなかったのですか？

坂井 沖縄100号の3回照射では——すべての区ではありませんが——、平均値はやや高くなっています。九州38号では、はっきりした一定の傾向は見られません。変異の偏りは余りなかったようです。

蓬原 別の問題ですが、栄養体の量的形質の場合には、³²Pよりも³⁵Sの方がよいような気がしますが、この点について何か実験データはないでしょうか。

坂井 ³⁵Sについては、データを持ち合わせていません。

河合 坂井氏は処理2年目栄養差で選抜してもよいとの意見でしたが、そのように考えて
よいでしょうか。
坂井 切千歩合の伝達力は高いようで、処理2年目栄養系でおおまかに選抜できます。しかし収量はそのような若い年代ではだめだと思います。
河合 処理2年目のイモのキメラの大きさについてはどう考えていますか。
坂井 その点については、まだ実際に観察していませんが、将来は問題にすべきことだと思います。
河合 キメラの発生は、栄養繁殖植物の突然変異育種で常につきまとう問題ですが、この点についての御意見をいただきたい。
中島 キメラは多数の細胞からなる生長点を照射する場合必然的に発生するものです。もし非常に少数の細胞から構成されている生長点を狙う場合には、突然変異は極めて少ないはずです。しかし分離可能なほどに大きいセクターの形で発生するものと考えます。だから技術的には、そのような生長点を持った芽がどこにあるかを見付けて、しかもどのようにしてその芽を生長させるかを考えねばなりません。
Asseyova（1927）がジャガイモで、普通の芽をすべてえぐり取り不育芽を生長させて、キメラでない突然変異を得た実験などは非常に参考になるものと思います。
広部 別の問題になりますが、サツマイモの場合に、内部照射をしないものにも選抜の効果が認められ、一方処理をしたのに効果があらわれなかったことについての解釈をお聞かせください。
私が数年前にカイコでおこなった実験でも、異常照射を行なって後代を選抜していくと、照射しない系統に最も選抜の効果が認められ、また照射系統でも低線量のものほど選抜が効いていました。
坂井 さしあがるもの場合は、処理、選抜の繰り返しにより変異が認められております。しかし、また選抜の効果は無処理区でもみられています。この実験では処理による変異を有効に選抜できなかった点が問題であります。量的形質の突然変異体を選抜することが将来の課題と考えております。
河合 今までの討議の内容を要約いたしますと；
1) 高等生物では、内部照射の場合のtransmutationの効果は、放射線の効果に比べてはるかに小さいと考えられます。
2) 個体レベルでは、内部照射では低線量率、長時間の照射が行なわれるため問題が複雑になっていたようです。
したがって、内部照射を用いたtransmutationの効果によって高等生物での突然変異率を高めることは期待できないということになります。
突然変異率について、照射の方法によって差がないという結果や、内部照射の方がより効果的であるという意見はもちろんですが、この点については今後の研究が待たれます。
3) サツマイモの実験では澱粉含量についての突然変異がおこること、切千歩合は処理3年目栄養系のいも単位でも選抜ができること、しかし、処理3年目以降の群単位に選抜を行なう方法が有効であること、さらに隔代に照射して選抜することを考えるのがよいというように、まとめることができましょう。
CHEMICAL MUTAGENESIS IN HIGHER ANIMALS

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Chemical mutagenesis is recently being applied widely to breeding especially in the field of plant breeding. And, although the usefulness of mutation breeding appears to be the chief point of contention at present in breeding, mutation breeding—either by chemical substances or by radiations—will in the future be more completely interwoven with the so-called conventional methods which are now too often considered as rivals. This point has already been mentioned by Mackey (1961).

I have been requested to review the present situation of chemical mutagenesis in higher organisms which provide considerable information on its application to breeding in this symposium. However, since an adequate review of such a broad and rapid growing area of research, and to explain the principal points of the subject in a short time require first hand experience which I do not have, I will describe only the results of some experiments which are believed to be suggestive for application in breeding among the works done by our group using Drosophila and the silk-worm. Several of these have already been published elsewhere.

First, we will consider the purpose of the experiments on chemical mutagenesis using higher organisms as the materials since it is well known that microorganisms are more suitable materials for detail analysis. We believe that the main purposes of chemical mutagenesis using higher organisms are as follows: 1) to find a clue for clarifying the fine structure of the chromosomes which can be observed by light microscope, that is, to reveal the molecular models for organization of DNA into chromosomes of higher organisms; 2) to determine the relative frequency patterns of mutations, chromosomal abnormalities of various types, lethals, translocations, induced crossing-over, etc., which are useful for application in breeding experiments; and 3) to test whether or not there is decisive specificity between the chemical structure of the agents and the genetic loci under tests. But, in higher organisms, the third item of specificity often sinks us in a bog unless we give warning in advance with respect to which matter in what level we want to determine specificity, and also inquire into the extent of influences of the secondary factors such as the position of the tested loci on the chromosomes, whether it masks or exaggerates or the specificity itself originated partly from the secondary
factors. For further detail discussion on these points, the readers can refer to Auerbach (1963).

There are also several technical difficulties in experiments on chemical mutagenesis in higher forms. First, there is the difficulty of the genetic materials in the higher organisms being safeguarded deep in the body, and in order to reach the genetic materials, chemical agents under tests must be able to penetrate into the body without decomposition by body fluid and to reach the nucleus of the cells of germ line. Secondly, the specificity with respect to the mutagenic effects of the tested substances must further reach a certain level in relation to other effects on the cell such as toxicity to the cytoplasm or nucleus, or the production of chromosomal disturbances leading to the death of the cell and consequently, to the mutated gene. These were also pointed out by Ehrenberg (1960).

1. The Significance of Cross-Linking in Chemical Mutagenesis

The alkylating substances with effective carcinostatic action generally possess two or more functional groups, although the possession of only one functional group is sufficient for mutagenesis or carcinogenesis. This is the opinion of many investigators in the fields of chemical carcinogenesis, chemotherapy of cancer and related subjects. It can be expected from this idea that the relative effectiveness of agents which have different functional groups would differ in frequencies of induced mutations and translocations. That is, it can be expected that polyfunctional alkylating agents are much superior to monofunctional alkylating agents in induction of translocations at the same concentration level to induce the same frequencies of lethal mutations. This fact is very helpful in application of chemical mutagenesis to animal and plant breedings. This subject was at first suggested by Dr. Auerbach, Institute of Animal Genetics, Edinburgh. We at first compared the frequencies of sex-linked recessive lethals and translocations in the progeny of Drosophila ♀♂ injected with either ethylene oxide which has only one functional arm or diepoxybutane which has two functional arms (Nakao and Auerbach, 1961). In this experiment, genetically detected translocations involving the Y and two large autosomes were used as a measure of chromosome breakage and the effective dose was estimated from the frequency of simultaneously induced sex-linked lethals. In other word, the relative chromosome-breaking ability of these two compounds was expressed by the ratio of translocations to lethals on the assumption that ethylene oxide is a less efficient chromosome breaker than diepoxybutane and it would be expected that the translocation/lethal ratio will be higher after treatment with diepoxybutane. This type of comparison can be used even for substances which resemble the two used here, which differ consider-
CHEMICAL MUTAGENESIS IN HIGHER ANIMALS

Table I. Mutagenic alkylating agents, their structures and their efficiencies in *Drosophila melanogaster*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>% Lethals</th>
<th>Molar conc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETHYLENE OXIDE</td>
<td>1.18</td>
<td>0.055</td>
</tr>
<tr>
<td>DIEPOXYBUTANE</td>
<td>5.32</td>
<td>0.09</td>
</tr>
<tr>
<td>ETHYLENE IMINE</td>
<td>6.52</td>
<td>0.005</td>
</tr>
<tr>
<td>TEM</td>
<td>11.82</td>
<td>0.0065</td>
</tr>
<tr>
<td>EMS</td>
<td>14.17</td>
<td>0.013</td>
</tr>
<tr>
<td>MYLERAN</td>
<td>7.86</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

rably in effectiveness on molar basis (Table I).

As the second experiment in this series of study, we compared ethylene imine which has only one functional arm and 2:4:6-tri-(ethyleneimino)-1:3:5-triazine (TEM) which has three functional arms in a similar manner (Nakao, Yamaguchi and Machida, 1964).

We will mention briefly the method used for both experiments. The alkylating agents were made up to different concentrations in 0.4% NaCl and injected into adult ♀♂ of a wild type strain (OrR), which is conventionally used for mutation experiments in Edinburgh and also in our laboratory. On the day following treatment, the ♀♂ were mated to ♀♀ of the genotype *y sc*<sup>St</sup> *In 49 sc*<sup>8</sup>; *bw*; *st*. Each ♀♂ was provided with two or three virgin ♀♀ every three days to produce successive broods. Lethals were scored in the progeny of the daughters crossed to Muller-5 ♀♂ and translocations were scored in the progeny of the sons mated to ♀♀ of the parental type.

The results of both experiments are summarized in Fig. 1, together with data obtained by X- and neutron-irradiation (Nakao and Machida, unpublished). Four triangles in Fig. 1 represent four experiments using ethylene oxide, diepoxynbutane and ethylene imine, and in other experiments only two broods were scored. Both
Fig. 1.
Lethals and translocations in three or two successive broods (1, 2, 3 or 1, 2) from *Drosophila* ♀ ♀ injected with ethylene oxide, diepoxybutane, ethylene imine or TEM. The data for X-ray and neutron irradiation are added.

lethal and translocation frequencies were determined by constructing the centre of gravity for both triangles and straight lines. From the figure, it was apparent that the ratio of translocations to lethals were the same after ethylene oxide and diepoxybutane treatments and the ratio was smaller in the treatment with TEM than in ethylene imine treatment. These facts lend no support to the idea that
the difference in carcinostatic activity between monofunctional and polyfunctional alkylating agents is due to cross-linking action of the latter type of compounds on the chromosome, which was the basic idea of this experiment.

It was also shown that in both cases of ethylene oxide versus diepoxynbutane treatments and ethylene imine versus TEM treatments, the alkylating agents with polyfunctional groups did, in fact, induce fewer translocations that can be expected from the square dose-effect law for large rearrangements.

We would also like to call your attention to the fairly large difference in the size of the triangles. This is perhaps due to the difference in sensitivity of the germ cells in different stages of spermatogenesis to the agents, or this may have originated from secondary factors such as the differences in extent of penetration of the agents to the germ cells of the different developmental stages, or the differences in chemical conditions of the cytoplasm which decompose the injected agents.

Thirdly, we tried to compare ethylmethane sulfonate and busulphan (myleran) but since several commercial samples of busulphan were ineffective for inducing lethals and translocations in the concentrations tested, perhaps due to the long inadequate storing of the agents in the drugstore, results have not been obtained as yet.

Whether the difference in chromosome-breaking ability between monofunctional and polyfunctional alkylating agents is due to the cross-linking action and whether this phenomenon is generalized in other cases remain to be studied in the future.

II. Possible Tests of Mutation-Induction with Metabolic Analogues of DNA by the Method of Axenic Synthetic Medium Feeding in Drosophila

In microorganisms, it is known that the metabolic analogues of DNA can induce mutations (Benzer and Freese, 1958; Freese, 1959a, 1959b; Litman and Pardee, 1956, 1960; Strelzoff, 1962). In the cells of higher organisms, it is also well known that the incorporation of the metabolic analogues can enhance the radiosensitivity of the cells. From these data it would be very probable and interesting to find out whether metabolic analogues can induce mutations even in higher organisms as well as in microorganisms. We tried to test the possible mutagenic action of several metabolic analogues (Nakao et al., 1964) and the results of such experiments will probably provide some hints on the fine structure of the chromosomes in higher organisms, and also on the molecular mechanisms of chemical mutagenesis.

We used the axenic synthetic medium feeding method developed by Sang (1956), and often used by Alderson (1960a, 1960b, 1961) to analyse the mechanisms of formaldehyde-induced mutations in D. melanogaster. The reason for use
of the synthetic medium feeding is that it can be expected that there is possibility of finding the strains which require nucleotides in their nutrition. We tried to induce sex-linked recessive lethals and also visible mutations on X-chromosome.

The feeding method used in these experiments was the same as that used by Alderson. Therefore, readers who desire detail of the method can refer to his papers. The reason for use of the specific loci method simultaneously is to detect mosaic mutations which would possibly be induced by analogues. And the markers \( y w f \) and \( m \) on the X-chromosome were used.

Table II. Tests of mutagenicity of base-analogues using Muller-5 method, when treatment is for the entire larval life (Experiment 1)

<table>
<thead>
<tr>
<th>Analogues</th>
<th>Conc. (M)</th>
<th>No. of tested chrom.</th>
<th>No. of lethals</th>
<th>% Lethals</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>1604</td>
<td>2</td>
<td>0.125</td>
</tr>
<tr>
<td>2-Aminopurine</td>
<td>(2 \times 10^{-4})</td>
<td>1983</td>
<td>2</td>
<td>0.101</td>
</tr>
<tr>
<td>5-Bromouracil + Aminopterin</td>
<td>(2 \times 10^{-4})</td>
<td>None emerged from treated larvae</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results of these experiments are shown in Tables II~V. It is clear from the Tables that the metabolic analogues used in these experiments could not induce mutations in these concentrations. However, it is difficult to consider that the failure of mutation-induction was due to the insufficient concentration of the analogues, because in our experiments in which 5-BU of the concentration of \(2.5 \times 10^{-5}\)M (the concentration was only five fold of that in the Table III) was used, all hatched larvae died in the stage of 2nd or 3rd instar.

Table III. Tests of mutagenicity of base-analogues of DNA using Muller-5 method, when treatment is for entire larval life (Experiment 2)

<table>
<thead>
<tr>
<th>Analogues</th>
<th>Conc. (M)</th>
<th>No. of tested chromosomes</th>
<th>No. of lethals</th>
<th>No. of (F_1) sterile (\varphi \varphi)</th>
<th>% Lethals</th>
<th>% Sterile (F_1) (\varphi \varphi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-BU</td>
<td>(5 \times 10^{-4})</td>
<td>2361</td>
<td>5</td>
<td>48</td>
<td>0.211</td>
<td>1.992</td>
</tr>
<tr>
<td>2-AP</td>
<td>(5 \times 10^{-4})</td>
<td>2157</td>
<td>3</td>
<td>16</td>
<td>0.139</td>
<td>0.736</td>
</tr>
<tr>
<td>Mixture*</td>
<td></td>
<td>2364</td>
<td>0</td>
<td>15</td>
<td>—</td>
<td>0.630</td>
</tr>
</tbody>
</table>

* Mixture: Theobromine 1mg, Caffeine 1mg, Deoxyuridine 1mg and 2, 6-Diaminopurine sulphate 1mg in 50 ml of medium.

Therefore, we assumed that the analogues could at least enter the body and perhaps reach the germ cells, yet could not induce mutations in higher organisms. Quite recently I have heard from Dr. Alderson that he also tried to induce mu-
Table IV. Tests of mutagenicity of base-analogues of DNA using Muller-5 method, when treatment is for the entire larval life (Experiment 3)

<table>
<thead>
<tr>
<th>Analogue</th>
<th>Conc. (M)</th>
<th>No. of test chromosomes</th>
<th>No. of lethals</th>
<th>No. of sterile F₁ ♀ ♀</th>
<th>% Lethals</th>
<th>% Sterile F₁ ♀ ♀</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>2270</td>
<td>2</td>
<td>9</td>
<td>0.087</td>
<td>0.395</td>
</tr>
<tr>
<td>5-BU</td>
<td>5 × 10⁻⁴</td>
<td>3307</td>
<td>2</td>
<td>8</td>
<td>0.060</td>
<td>0.241</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3204</td>
<td>1</td>
<td>24</td>
<td>0.031</td>
<td>0.743</td>
</tr>
</tbody>
</table>

* F₀-test was conducted to examine fractional lethals (mosaic mutations for lethals).

Sex-linked recessive visible mutation test (y, w, m, f)

<table>
<thead>
<tr>
<th>Analogue</th>
<th>Conc. (M)</th>
<th>No. of F₁ ♀ ♀</th>
<th>No. of mutants (Whole-body)</th>
<th>Mutation freq.</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>20985</td>
<td>1</td>
<td>0.005</td>
</tr>
<tr>
<td>5-BU</td>
<td>5 × 10⁻⁴</td>
<td>9600</td>
<td>1</td>
<td>0.010</td>
</tr>
</tbody>
</table>

No fractional mutant was found in this experiment.

Table V. Tests of mutagenicity of base-analogues of DNA using Muller-5 method when the feeding of analogues is extended for the entire larval stages in *Drosophila melanogaster*. And the changes of radiosensitivity in mature spermatozoa by feeding with metabolic analogues

<table>
<thead>
<tr>
<th>Analogues</th>
<th>conc. (M)</th>
<th>No. of tested chromosomes</th>
<th>No. of lethals</th>
<th>No. of semi-lethals</th>
<th>No. of sterile lethals</th>
<th>No. of F₁ sterile</th>
<th>Mutation frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 Iodo deoxyuridine</td>
<td>5 × 10⁻⁴</td>
<td>1145</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>0.44 ± 0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ X-ray 1 kR*</td>
<td>957</td>
<td>19</td>
<td>10</td>
<td>3</td>
<td>3.03 ± 0.55</td>
<td></td>
</tr>
<tr>
<td>5 Iodo deoxyuridine</td>
<td>6mg/50ml</td>
<td>1120</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>0.18 ± 0.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1022</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0.39 ± 0.20</td>
<td></td>
</tr>
<tr>
<td>Sulfinouracil</td>
<td>5mg/50ml</td>
<td>1915</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>0.26 ± 0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+ X-ray 1 kR*</td>
<td>1599</td>
<td>36</td>
<td>6</td>
<td>6</td>
<td>2.63 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>2 Thio 4,6 dioxypyrimidine</td>
<td>1,3 Dimethyl uracil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mutation frequencies include semi-lethals.

* indicates the brood II of each three days. And no sign indicate the brood I. Mutation frequency in used strain (OrR) is 2.69 ± 0.54 (brood I) and 2.99 ± 0.60 (brood II) with X-irradiation (1 kR).
tations by metabolic analogues but did not succeed (private communication).

On the other hand, there is a paper in which it is reported that BUdR will induce sex-linked lethals but will not induce any chromosomal rearrangement in *D. melanogaster* (Kaufmann and Gay, 1963). Although the results of these experiments, including ours, cannot be said to be conclusive, backed by positive results, it is our idea that the structure of the chromosomes of higher forms is not as simple as in the case of microorganisms. For further information on the fine structure of the chromosome, more detail experiments are required.

**III. Comparisons of the Mutagenic Effects of Various Nitrogen Mustard Derivatives**

It is generally thought that the chemical mutagens belonging to the same chemical groups can induce mutations by similar mechanisms. This concept is well established with certain groups of chemicals in case of microorganisms, such as the induced mutation by base analogues by Freese (*loc. cit.*). He postulated that the base analogues 2-aminopurine and 5-bromouracil induce transitions, that proflavin induces transversions. From this information, vague hope of inducing mutations to apply on breeding gave us the impression that resemblance in chemical structure means the corresponding strength of mutagenic actions of these substances.

However, in higher organisms, the circumstances are not so simple, since each agent must, as mentioned in the beginning, meet various barriers such as body fluid, cellular membranes and cytoplasmic substances which often decompose the agents before reaching the goal.

Therefore, if we wish to use some chemical mutagens in breeding experiments, we must try to know the optimum concentration of each substance even if we already know the appropriate concentration for mutation-induction with an agent of similar series.

Here, we would like to mention the results of the experiments which show the mutation frequency patterns with various concentrations of nitrogen mustard derivatives using silkworm visible mutations. The material and methods used in this study have been often described elsewhere (Nakao *et al.*, 1959), and only a brief outline will be given here. Pink eye (*pe*) and red eye (*re*) which are located on the chromosome V at 0.0 and 31.7, respectively, were used as the markers. Both control the colour of the egg (and also the colour of the eye in the adult). Whereas the normal egg colour is black, *pe*/*pe* is white and *re/re* is red. 0.05ml of each agent dissolved in 0.4% saline were injected into the abdomen of male wild moths, and about 20 minutes after the injection, these moths were mated to
<table>
<thead>
<tr>
<th>Derivatives of nitrogen mustards</th>
<th>Molar conc.</th>
<th>No. of eggs examined</th>
<th>Whole-body mutants</th>
<th>Mosaic mutants</th>
<th>Total mutated eggs</th>
<th>Mutation frequencies (%)</th>
</tr>
</thead>
</table>
| \(\alpha\)-(N-(bis-\beta^\prime\text{-chlo}
oxyethyl)-amino)-acetic acid hydrochloride | \(4.3 \times 10^{-3}\) | 2770 | 7 | 20 | 136 | 68 | 231 | 8.34 |
| | \(2.1 \times 10^{-3}\) | 7400 | 11 | 8 | 154 | 79 | 252 | 3.41 |
| N-(bis-\beta\text{-chlo}
oxyethyl)-N-(\beta^\prime\text{-ethoxy}
ethyl)-amine hydrochloride | \(3.1 \times 10^{-3}\) | 1920 | 14 | 3 | 89 | 70 | 176 | 9.17 |
| | \(1.6 \times 10^{-3}\) | 1664 | 0 | 1 | 29 | 19 | 49 | 2.95 |
| | \(0.8 \times 10^{-3}\) | 6554 | 3 | 1 | 131 | 74 | 209 | 3.19 |
| N-bis-\beta\text{-chlo}
oxyethyl-N-(\beta^\prime\text{-ethoxy}
ethyl)-amine-N-oxide hydrochloride | \(5.5 \times 10^{-3}\) | 3379 | 2 | 0 | 52 | 22 | 76 | 2.25 |
| | \(2.8 \times 10^{-3}\) | 6993 | 8 | 2 | 94 | 40 | 144 | 2.06 |
| N-(bis-\beta\text{-chlo}
oxyethyl)-benzylamine-N-oxide hydrochloride* | \(3.6 \times 10^{-3}\) | 4233 | 9 | 3 | 207 | 132 | 351 | 8.29 |
| N, N-diethyl-N'-(bis-\beta\text{-chlo}
propanediame-N,N'-dioxide dihydro
cloride | \(7.0 \times 10^{-3}\) | 11027 | 6 | 0 | 70 | 8 | 84 | 0.76 |
| | \(3.5 \times 10^{-3}\) | 11500 | 3 | 1 | 19 | 6 | 29 | 0.25 |
| | \(1.8 \times 10^{-3}\) | 11900 | 1 | 0 | 5 | 1 | 7 | 0.06 |
| N, N-diethyl-N'-(bis-\beta\text{-chlo}
propanediamine dihydro
cloride | \(1.5 \times 10^{-3}\) | 5923 | 78 | 18 | 534 | 495 | 1115 | 18.83 |
| | \(0.8 \times 10^{-3}\) | 12257 | 73 | 12 | 769 | 343 | 1197 | 9.77 |
| Control | \(14837\) | 2 | 0 | 5 | 0 | 7 | 0.047 |

i. The number of eggs examined is approximate number (exact number will be examined next spring, 1967).
ii. LD_{50}: Intraperitoneal dose in rats; MED: Minimum efficient dose=Minimum dose which caused the cytological effect upon the Yoshida Sarcoma cells.
iii. * silkworm from Saitama Sericultural Experiment Station and others from Chiba Sericultural Experiment Station.
double recessive female moths. The eggs from these matings were examined for their colours. The number of white or red eggs and mosaic eggs for white and red were expressed as proportion of the total number of pigmented eggs for the estimation of mutation frequencies. And the number of pigmented eggs are approximate at present because the exact number cannot be obtained until after they would hatch next spring.

The results are summarized in Table VI. And the table suggests the following points, 1) the effects of these nitrogen mustard derivatives tested on rat survivals or cytological damage upon the Yoshida Sarcoma cells differed considerably from their mutagenic efficiencies; 2) since the dose/effect relationships can be determined most reliably when it is analysed in a wide range of concentrations, and since the dose/effect relationships were tested within a limited range in this experiment, it is not possible to draw a definite conclusion. However, to the extent of the examination was made, the dose/effect relationships differed considerably with each substance; 3) the mutagenic action of these substances depends not only on the number of functional arms but also on other structures of the substances; 4) generally the oxidation of N depresses the mutagenic action of these derivatives.

For further analysis of the relationships between the structure of the chemical mutagens and their mutagenic action, readers can refer to recent reviews (Krieg, 1963; Orgel, 1963 and Loveless, 1966).

IV. Mutagenicity of N-Methyl-N’-Nitroso-N-Nitrosoguanidine in Drosophila and in the Silkworm

It is known that N-methyl-N’-nitroso-N-nitrosoguanidine (NG) is a very good chemical mutagen for microorganisms (Mandell and Greenberg, 1960; and Eisenstark et al., 1965), that is, this agent induces high mutations and very little lethals.

We tested the mutagenicity of this agent with Drosophila melanogaster and the silkworm by injection into adult flies and moths. NG dissolved into 0.4% saline solution at the concentration of 2.7×10⁻⁴M was injected into adult ♀♂ of a wild type strain OrR (8±5 hrs after emergence) which is generally used for mutation experiments. Sex-linked recessive lethals and autosomal translocations were detected as described in Experiment I. In the case of the silkworm moths, NG at concentrations 1.4×10⁻³M~1.4×10⁻⁸M was injected into the wild type moths and then they were mated to pe re/pe re females.

The results are shown in Table VII and Table VIII. From Table VII, it can first be noted that the frequencies of translocation are very low and that there seems to be fairly big scale cluster of lethal mutations as indicated in the foot-
Table VII. Sex-linked recessive lethals and translocations after treatment of adult \( \delta \delta \) with N-methyl-N'-nitroso-N-nitrosoguanidine

<table>
<thead>
<tr>
<th>Brood</th>
<th>LETHALS</th>
<th>TRANSLOCATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of tested chromosones</td>
<td>Lethals</td>
</tr>
<tr>
<td>I</td>
<td>1036</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>(1014)</td>
<td>(32)</td>
</tr>
<tr>
<td>II</td>
<td>949</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(904)</td>
<td>(8)</td>
</tr>
<tr>
<td>III</td>
<td>987</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>(963)</td>
<td>(9)</td>
</tr>
<tr>
<td>Total</td>
<td>2972</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>(2881)</td>
<td>(49)</td>
</tr>
</tbody>
</table>

Mutation frequencies include the semi-lethals.

The concentration of nitrosoguanidine was \( 2.7 \times 10^{-4} \)M, and each male of OrR wild strain is received 3\( \mu l \) of the solution.

There was fairly big scale clusters of mutations (No. 85 male), that is, 6 lethals in 8 (brood I), 6 in 10 (one of them was semi-lethal) (brood II), and 10 in 10 (one of them was semi-lethal) (brood III).

Numbers in parentheses: Clusters of mutations were counted as 1 mutation, and the sterilized were excluded from tested numbers.

Table VIII. Visible mutation frequencies induced by the treatment of adult \( \delta \delta \) with N-methyl-N'-nitroso-N-nitrosoguanidine (NG) in the silkworm

<table>
<thead>
<tr>
<th>Concentrations(M)</th>
<th>No. of eggs examined</th>
<th>Whole-body mutants</th>
<th>Mosaic mutants</th>
<th>Total mutants</th>
<th>Mutation frequencies(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 1.4 \times 10^{-3} )</td>
<td>17,346</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>( 2.7 \times 10^{-3} )</td>
<td>18,192</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>( 6.8 \times 10^{-3} )</td>
<td>23,004</td>
<td>7</td>
<td>0</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>( 1.4 \times 10^{-2} )</td>
<td>9,936</td>
<td>7</td>
<td>0</td>
<td>53</td>
<td>18</td>
</tr>
<tr>
<td>Control</td>
<td>22,041</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

\( i \) The number of eggs examined is shown in round numbers (exact number will be examined next spring).

\( ii \) 0.05mL of each solution of NG was injected into the male moths.

Note of the Table. Secondly, the brood pattern seems also to be of a special type, that is, lethal frequency is the highest in the 1st brood, while it is fairly low in the 2nd and 3rd broods when compared with that of the 1st brood. It is impossible
for us to examine the dose-effect relationship with NG, but the results of four experiments with different concentrations of NG using silkworm were shown in Table VIII. In these experiments the number of pigmented eggs which were basic number of calculation of mutation frequencies, is also shown in round number. However, the mutation rate at the concentrations of $1.4 \times 10^{-2}$M and $5.4 \times 10^{-3}$M were considerably high.

It is not possible for us to discuss further the characteristics of NG as a chemical mutagen in comparison with other substances such as nitrogen mustards, ethylene imine, TEM, EMS or so on, since the investigation of its mutagenic actions has not been completed as yet. However, as far as we know, NG cannot be considered as being an excellent mutagen in higher organisms.

CONCLUSION

What we can conclude from these fragmentary results on the subject or object of the experiments on chemical mutagenesis using higher organisms? It is, of course, quite impossible to explain the fine structure of chromosomes cn the basis of DNA molecules from this kind of experiments. It may only be possible to find a clue for discussing whether the hypothetical models of the fine structure of the chromosomes already proposed can be confirmed by experimental results or not. That is to say, these experiments cannot positively reconstruct the new models but can negatively point out whether the models already presented can fit or can explain the fact proved experimentally or not. Of course, we must try to design experiments in which we can build a good model of the fine structure of the chromosome.

It is suggested from these experiments that the chromosomes of the higher organisms do not exist in the simple form of DNA which has already been established in the microorganisms, since experiments I and II showed that the cross-linking was not proved and also that metabolic analogues could not induce mutations. Needless to say, further experiments are necessary to conduct before a definite conclusion can be drawn.

With regard to the relative frequency patterns of mutations, translocations, and other chromosomal changes, it was suggested that the patterns would vary depending upon the chemical mutagens used and also depending upon the biological materials or genetic markers employed.

And finally, it was shown that carefully designed preliminary experiments in advance would be the shorter way for success in applications of chemical mutagenesis in breeding.
ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Dr. C. Auerbach, Institute of Animale Genetics, Edinburgh for her valuable advices and suggestions rendered during the course of these works.

Some of these studies were supported partly by a grant-in-aid from the Ministry of Education of the Japanese Government and also by a research grant from the Tōyō Rayon Foundation for the Promotion of Science and Technology.

References

山口

Cross-linking の問題について質問します。EI 系統のアジェンシ系化合物について、昆虫において不妊剤として作用するもの、二つあるものが使用されておりますが、そのような化学物質にかんする実験を計画していますか。

仲尾
現在、アジェンシ系化合物の実験計画はありません。cross-linking について研究室の中西氏が EI や TEM を培養細胞に与えたところ、転座のような染色体像は polyfunctional arms のアルカリ化合物でしか起らないことを認めております。このように細胞学的には cross-linking 説の方が正しいような点もありますから、私の実験で得られた結果を一般化するとは思っておりません。

山口
化学物質がどれくらい DNA の中にとり込まれたかを証明するにはどうすればよいのでしょうか。

仲尾
それは私達も常に疑問に思っている問題です。現在、化学物質が遺伝物質に直接どういったことを証明するデータを持っておりませんが、将来は標識化合物をその目的のために使用するつもりです。

山口
微生物の実験によると、＝トロソアビジシオンは体内に入ってジアゾルカンになって働くというデータがありますが、その点についてのお考えを聞きたいです。

仲尾
たとえばシュージョウバエでは、ホルムアルデヒドがそのままの形で突然変異を起こさせるのではなく、蛋白と結び付いた中間的な形として働くっているというデータがあり、微生物の実験に限らず、どの実験においても、化学物質が体内でそのまま働くのかどうかは体内にどのようなものと組合して中間的なものとなって働くのかを重要と考えています。そのためには、化学者との共同研究が必要であると考えています。
COMPARISON OF MUTAGENIC EFFECTS OF ETHYLENE-IMINE AND IONIZING RADIATIONS ON RICE

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Faculty of Agriculture, The University of Tokyo, Japan

Recently, it has been found that alkylating compounds, like ethylene-imine (EI) and ethyl-methane-sulfonate (EMS), lead to higher mutation rate and bear mutagenic specificity when it was compared with ionizing radiations. In plants, however, as research was mainly conducted with regard to induced chlorophyll mutations, there remains a paucity of available information whether they would be valid with regard to induced field mutations with economic importance or not.

The purpose of this paper is to present the data obtained in our laboratory on the mutagenic effects of EI, X-ray and thermal neutron (Nth), especially about the induced field mutations.

Dry seeds of rice 'Norin, No. 8', which were maintained to contain approximately 12 percent moisture, were used as the material. The seeds were treated in an aqueous solution containing EI at pH 7 followed by washing with tap water for 24 hrs. X-rays were produced by a 190 kV machine operated at 25 mA and passed through 1.0 mm Al-filter. Thermal neutron was generated in the thermal column of the nuclear reactor (JRR-1) of the Tokai-Laboratory, Japan.

Fig. 1 Dosage effects on survival rate and fertility in M₁-generation and chlorophyll mutation rate per panicle progeny in M₂-generation.
1. \( M_1 \) Lethality and Fertility

Fig. 1 shows \( M_1 \) survival rates and fertility percentage obtained from the investigation at the time of the harvest. In the figure, doses of each mutagenic agent were marked so that the doses of the same lethal effects correspond with them. The lethal effect of EI at a dose of 0.1 per cent for 2 hrs. is equivalent to that of X-ray at a dose of 20 kR. The figure shows that X-ray produces more pronounced decrease in \( M_1 \)-fertility than EI at doses inducing the same lethal effects. This is of interest because X-ray produces higher rates of \( M_2 \)-partial sterility which could be caused by chromosomal aberrations, as will be mentioned later.

2. \( M_2 \) Mutation Rates

Chlorophyll mutation rates per \( M_2 \) panicle progeny are shown in Fig. 1. They were determined by using the \( M_2 \) panicle progenies which include more than 30 seedlings in each. It is clear from the figure that EI indicates approximately twice as high \( M_2 \) chlorophyll mutation rates as those obtained with X-ray and thermal neutron. This agrees closely with the results obtained by Ehrenberg et al. (1958) in barley.

The investigation of field mutations has been done to the \( M_2 \) panicle progenies after treatments with 0.7 per cent of EI, 20 kR of X-ray, and \( 3.6 \times 10^{10} n/cm^2 \) of thermal neutron. The former two treatments have similar \( M_1 \) lethal effects in each, and the latter one has half the \( M_1 \) lethal effect of them (see Fig. 1). In the field, 15 plants were grown in each \( M_2 \) panicle strain. The mutations concerning quantitative characters were screened on the basis of twice the standard deviation from the mean of the control population. For the field mutation which were screened in the \( M_2 \) generation, the progeny tests were pursued in the \( M_3 \) generation.

The types of \( M_2 \) chlorophyll and field mutations and the mutation rates per \( M_2 \) panicle progeny obtained from the investigation are listed in Table 1. It can be seen from the table that though EI indicates the highest chlorophyll mutation rate among the mutagenic agents, this situation is not always applicable to other field mutation rates. This is more clarified in Table 2 which indicates the relation between the rates of chlorophyll mutation and other field mutations by applying sign-test to the data of Table 1. It is remarkable that the rate of partial sterility, which seems to be caused chiefly by chromosome translocation, is more than twice high in X-ray than in EI contrary to the rate of chlorophyll mutation (see Table 1). Similar results have also been obtained by Ehrenberg et al. (1959, '60) for barley.
Table 1. Types of mutations and the mutation rates per M₄-panicle progeny

| Mutagen | No. of M₄ -panicle progenies | Chlorophyll mutation | Sterility | Morphological-
|---------|-----------------------------|----------------------|-----------|-----------------
|         | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   | No. | %   |
| EI      | 467 | 13.1 | 42  | 9.0 | 24  | 5.1 | 127 | 27.2 | 46  | 9.9 | 23  | 4.9 | 69  | 14.8 | 17  | 3.6 | 16  | 3.4 | 19  | 4.1 | 17  | 3.6 |
| X-ray   | 339 | 6.5  | 5   | 1.7 | 12  | 3.5 | 39  | 11.5 | 29  | 8.6 | 43  | 12.7| 72  | 21.2 | 6   | 1.8 | 6   | 1.8 | 9   | 2.7 | 14  | 4.1 |
| N₄k    | 543 | 3.1  | 6   | 1.1 | 11  | 2.0 | 34  | 6.3  | 11  | 2.0 | 17  | 3.1 | 28  | 5.2  | 7   | 1.3 | 5   | 0.9 | 5   | 0.9 | 4   | 0.7 |
| Total   | 1349| 7.4  | 53  | 3.9 | 47  | 3.5 | 200 | 14.8 | 86  | 6.4 | 83  | 6.2 | 169 | 12.5 | 30  | 2.2 | 27  | 2.0 | 33  | 2.4 | 35  | 2.6 |

Table continued...

<table>
<thead>
<tr>
<th>Colored glume</th>
<th>Heading-time mutation</th>
<th>Culm-length mutation</th>
<th>Panicle-length mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>8</td>
<td>1.7</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>11</td>
<td>3.2</td>
<td>7</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>0.7</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>23</td>
<td>1.7</td>
<td>11</td>
<td>0.8</td>
</tr>
</tbody>
</table>

1) EI: (0.7% sol. 2hrs), X-ray: (20 kR), N₄k: (3.6×10¹⁴ n/cm²)
Table 2. Relation between the rates of chlorophyll mutation and other mutations

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Significance 1)</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>**</td>
<td>The rate is high for chlorophyll mutation, but not so high for other mutations</td>
</tr>
<tr>
<td>X-ray</td>
<td>**</td>
<td>The rate is high for chlorophyll mutation, but high for other mutations</td>
</tr>
<tr>
<td>NaH</td>
<td>N. S.</td>
<td>No relationship between the rates of chlorophyll mutation and other mutations</td>
</tr>
</tbody>
</table>

1) Inference from sign test applied to the data of Table 1.

Then, the mutations which were most mutagenic to EI and to X-ray were classified by applying $\chi^2$-test to the data of Table 1 by using the ratios of EI: X-ray: NaH of chlorophyll mutation and partial sterility respectively as theoretical rates. The results are shown in Table 3. It is apparent in the table that some mutations of morphological characters, like dwarf, slender type, compactoid, and uni-culmis, are as mutagenic to EI as chlorophyll mutation, whereas the mutations of quantitative characters, like heading-time, culm-length and panicle-length, are either most mutagenic to X-ray like partial sterility or equally mutagenic to X-ray and EI. These imply that different mutagenic agents bear different specific actions. Furthermore, the fact that X-ray induced a higher rate of partial sterility leads to the suggestion that the mutations which are most mutagenic to X-ray include also those caused by the induction of chromosomal aberrations.

Table 3. Classification of mutations according to mutagenic agents

<table>
<thead>
<tr>
<th>Mutations most mutagenic to EI</th>
<th>Mutations most mutagenic to X-ray</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll</td>
<td>Partial sterility</td>
<td>Complete sterility</td>
</tr>
<tr>
<td>Dwarf</td>
<td>Colored glume</td>
<td>Awned</td>
</tr>
<tr>
<td>Slender type</td>
<td>No heading</td>
<td>Early heading</td>
</tr>
<tr>
<td>Compactoid</td>
<td>Late heading</td>
<td>Short culm</td>
</tr>
<tr>
<td>Uni-culmis</td>
<td>Long culm</td>
<td>Short panicle</td>
</tr>
<tr>
<td></td>
<td>Long panicle</td>
<td></td>
</tr>
</tbody>
</table>

3. Mutation Spectra

Another indication of mutagenic specificity can be cited from the differences observed in mutation spectra among mutagenic agents. Table 4 and the data of Table 1 indicate that (1) with regard to the chlorophyll mutations, the proportion of viridis is larger in EI than X-ray, (2) as for the sterilities, the proportion of
partial sterility is larger in X-ray and thermal neutron than EI, (3) with regard to the heading-time, the proportion of late-heading mutations is larger in X-ray and thermal neutron than EI, and (4) as for the culm-length and panicle-length, no significant differences of mutation spectra exist among the mutagenic agents.

Table 4. Differences in mutation spectra among mutagenic agents

<table>
<thead>
<tr>
<th>Significance</th>
<th>Within chlorophyll mutation (Albina: Viridis: Others)</th>
<th>Within sterilities (Complete : Partial)</th>
<th>Within heading-time mutations (Early : Late)</th>
<th>Within culm-length mutations (Short : Long)</th>
<th>Within panicle-length mutations (Short : Long)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI -x-X</td>
<td></td>
<td>EI -y-X</td>
<td>X</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>EI -y -Nth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1) Inference from heterogeneity-test applied to the data of Table 1.

The observation that the spectrum of chlorophyll mutations varies with mutagens and the fact that EI produces more viridis mutants when compared to those of X-ray have also been reported by Ehrenberg et al. (*loc. cit.*) in barley, as well as McKelvie (1963) in Arabidopsis.

Recently, the locus distribution of mutants induced by mutagenic agents has been analyzed suggesting a specificity of chemicals, like EI and EMS, on individual gene loci (Favret, 1960 a, -b, Lundqvist et al, 1961, and McKelvie, *loc. cit.*). This makes an assumption possible that the action of such chemicals is restricted to certain small regions of chromosomes in contrast to the random action of ionizing radiations.

4. Simultaneous Appearance of Mutations

Frequently, mutations appear simultaneously for two or more different characters in a M<sub>2</sub>-plant. In order to know whether some dependency exists among the

Table 5. Distribution of mutant characters per M<sub>2</sub>-plant

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>No. of M&lt;sub&gt;2&lt;/sub&gt;-panicle progenies</th>
<th>Percent of M&lt;sub&gt;2&lt;/sub&gt;-panicle progenies with indicated number of mutant characters per plant</th>
<th>Heterogeneity test</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>467</td>
<td>Exp. Obs.</td>
<td>70.9 25.8 3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>76.2 16.7 7.1</td>
</tr>
<tr>
<td>X-ray</td>
<td>339</td>
<td>Exp. Obs.</td>
<td>54.1 36.2 9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>59.6 27.1 13.3</td>
</tr>
<tr>
<td>N&lt;sub&gt;th&lt;/sub&gt;</td>
<td>543</td>
<td>Exp. Obs.</td>
<td>84.9 14.1 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>89.5 6.3 4.2</td>
</tr>
</tbody>
</table>
mutations or not, the observed and expected distributions of mutant characters per M₃-plant were compared as shown in Table 5. Four characters, sterility, heading-time, culm-length, and panicle-length were used, and expected distributions were calculated from the mutation rates of the four characters indicated in Table 1, according to the Null hypothesis each character of that mutates independently. The results indicate the significant differences between the observed and expected distributions and hence it may be understood that there exists some dependency among mutations of those four characters after both treatments with EI and ionizing radiations.

In Table 6, the distribution of mutant characters per M₃-plant was compared between M₃-groups classified by induction of chlorophyll and non-chlorophyll mutations. As can be seen in the table, a significant difference in the distribution was observed between the two groups after the treatment with EI in contrast to the treatments with ionizing radiations. This implies that the chlorophyll mutation induced by EI has a tendency to be attended by more other mutations.

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>M₃-groups</th>
<th>Numbers of M₃-panicle progenies with indicated number of mutant characters per plant</th>
<th>Heterogeneity test</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>Non-chloro. mut. group</td>
<td>246 61 15 340</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Chloro. mut. group</td>
<td>92 17 18 127</td>
<td></td>
</tr>
<tr>
<td>X-ray + Nᵣα</td>
<td>Non-chloro. mut. group</td>
<td>636 114 59 809</td>
<td>N. S.</td>
</tr>
<tr>
<td></td>
<td>Chloro. mut. group</td>
<td>52 12 9 73</td>
<td></td>
</tr>
</tbody>
</table>

This tendency was confirmed by the comparison of the proportion of mutant type to normal type for each of those characters among chlorophyll and non-chlorophyll mutation groups. It was apparent from Table 7 that the chlorophyll mutation induced by EI is apt to be accompanied by the heading-time and panicle-length mutations. The chlorophyll mutation induced by ionizing radiations is accompanied by the heading-time mutation.

The above mentioned dependency among mutations may be caused not only by the pleiotropic effects of a mutant gene, but also by the simultaneous changes
Table 7. Difference in induction of mutations between $M_3$-groups classified by induction of chlorophyll and non-chlorophyll mutations

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>$M_3$-groups</th>
<th>Culm-length</th>
<th>Panicle-length</th>
<th>Heading-time</th>
<th>Sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>Non-chloro. mut. group</td>
<td>293</td>
<td>47</td>
<td>327</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Chloro. mut. group</td>
<td>103</td>
<td>24</td>
<td>116</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Heterogeneity test</td>
<td>N. S.</td>
<td>**</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>$X + N_{th}$</td>
<td>Non-chloro. mut. group</td>
<td>713</td>
<td>96</td>
<td>766</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Chloro. mut. group</td>
<td>66</td>
<td>7</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Heterogeneity test</td>
<td>N. S.</td>
<td>N. S.</td>
<td>**</td>
<td></td>
</tr>
</tbody>
</table>

of two or more genes located in a small region of chromosomes, resulting from the induction of linked mutations or chromosomal deficiency and duplication. On this point further research would be required.

References

イネに対する Ethylene-imine と電離放射線との
突然変異誘発効果の比較

大曾根兼一
（東京大学農学部）

近年，EI や EMS のような化学物質が電離放射線に比べて，高い突然変異率と特異な作用性を持つことを示唆するいくつかの報告がなされてきた。しかし，それらの多くは葉緑変異を対象としたものであり，他の field mutations については，さらにデーターを積み重ねる必要がある。そこで，特にイネの field mutations を中心として，われわれの研究室で得られた結果を報告する。

水稻農林 8 号の気乾種子（水分含有約12％）を用い，EI 処理は pH 7 の水溶液として 2 時間発泡後 2 時間水洗し，電離放射線処理は X 線 (190 kV, 25mA, 1,000R/min., 1.0mm Al-filter)，および NaK（JRR1，第 7 実験孔）を用いて種子照射を行なった。

1. M1 致死および稔性
（1）M1 生存率で比較した場合，EI の 0.1％ 2 時間処理は X 線の 2.5 kR 照射に相当した（Fig. 1）。
（2）同じ生存率を示す線量で，X線は EI よりも高い稔性障害を示した （Fig. 1）。

2. M2 突然変異率
（1）field mutations の調査は，M1 致死効果のほぼ等しい 0.7％ EI 区および 20kR X 線区と，それらの約半分の M1 致死効果をしぼした 3.6×10^5n/cm² NaK 区の 3 区に限定した。M3 確定系統は 15 種類（稲穂変異調査は 30 種類以上）とは，突然変異の選抜は特に分離に注目するとともに，量的形質については標準区の信頼限界を基準として行ない，さらに M3 検定によって確認した。Table 1 は，このようにして得られた M2 突然変異の種類と頻度を示す。
（2）EI は電離放射線の約 2 倍の葉緑変異率を示したが（Fig. 1 および Table 1），その他の形質の突然変異率は必ずしも高くなく（Table 2），矮性，細粒，密粒，および無分けなどの一部の形質変異を除いては，X線と同等か，それよりも低い値を示した（Table 3）。これらの結果は各 mutagen における特異な働きが存在することを示す。
（3）X 線処理区で部分不稔の出現が大であったことは（Table 1 および 3），X線で起こり易い突然変異（Table 3）に，栄養体異常に起因するものがより多く含まれている可能性を示唆する。

3. 突然変異スペクトル
（1）mutagens による特異な作用性の存在は，葉緑，稔性，および出穂期の各変異のなかで突然変異スペクトラムの差からも知ることができる（Table 4）。
（2）EI 処理区では葉緑変異のなかで特に viridis の比率が高く，一方，電離放射線処理区は，稔性変異のうち部分不稔が，また出穂期変異のうち晚生が，それぞれ，より高い
比率で現れた（Table 1 および 4）。

4. 突然変異の同時的出現

(1) 種性、出穂期、穂長、および穂長の 4 形質について、M2 個体当たりの変異形質数の頻度分布が調べられた（Table 5）。

(2) いずれの mutagens においても、1 個体のなかで2つ以上 の形質が同時に変異を生ずる頻度は、各形質が独立に変異すると仮定して確率論的に期待される頻度よりも大きく、従って、各形質の変異発生の間に何らかの関連性が存在することが推定される（Table 5）。

(3) 特に EI 処理区では、葉緑変異系および非葉緑変異系に比べて、他形質の変異を一層伴ない易い傾向があり（Table 6），出穂期変異と穂長変異がより高い頻度で出現した（Table 8）。

(4) このような形質間の変異発生の関連性には、pleiotropy, linked mutations, 染色体の欠失、重複による場合などが考えられるが、これらの点については今後の研究に待たなければならない。
MUTAGENIC EFFECTS OF SEVERAL ALKYLATING
SUBSTANCES IN RICE WITH REFERENCE
TO THE METHOD OF TREATMENT

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As pointed out by Auerbach (1961), chemical mutagenesis has been investigated mainly from two different stand-points, i.e., hunting for more effective mutagens and application of the newly detected mutagens in the field of nucleic acid chemistry. For the progress of breeding, however, it is further necessary to elucidate the mutagenic processes or effects biologically. It will need to form the third field of investigating chemical mutagenesis. As the first step of the biological approach, the mutagenic characteristics of known mutagens should be elucidated in relation to the direct response of genetic materials.

From this viewpoint, a series of experiments has been conducted on rice since 1963, in which several alkylating substances have been compared. The results will be reported in two parts.

1. Mutagenic Characteristics

Six alkylating compounds, i.e., ethyleneimine (EI), ethylene oxide (EO), diethylsulfate (DS), methyl methanesulfonate (MMS), ethyl methanesulfonate (EMS) and propyl methanesulfonate (PMS) were used in 1963. Dry seeds of a rice variety Gimbôzu were treated with solutions of those chemicals at various concentration (0.01~1.0%, v/v) and with X-rays (5~50kR) for comparison. Treatments with the chemicals were conducted for 2.5 hr at 17~18°C for EI, for 17 hr at 17~18°C for EO, 2 hr at 17~18°C for DS, and 17 hr at 20~21°C for MMS, EMS and PMS.

Chlorophyll mutations were induced by all agents except MMS. Within the limit of the dosages given in this experiment, the maximum rate of chlorophyll mutation was obtained by EI treatment at about its LD_{50}-dosage. The rate was 33.3% per M₁-panicle progeny and 4.83% per M₂ seedlings. The most effective dosage for other chemicals could not be determined. However, the mutagenic effect of EMS and PMS seemed to be larger than that of X-rays, as reported by Yamagata et al. (1965).

On the basis of the M₁ data, several doses of four agents (EI, EMS, PMS and X-rays) were chosen for observation of the M₂ generation and the mutations on
agronomic characters, mainly on heading date, were investigated.

Heading date of aberrant M₂ plants was in the range of ten days earlier and about fifty days later than that of the original variety (Aug. 31), regardless the kind of agents. Relative frequency of early-type to late type mutants (in a broad sense) was about 3 : 7 in all agents. Thus, no difference in mutation spectrum could be found so far as the heading date is concerned.

As for the total frequency of mutants on heading date, dosage-response was different among four agents, as it is shown in Fig. 1. From this result, it can be said that the mutagenic effect of four agents is in the order of EI\(\gg\)EMS\(\gg\)PMS\(\gg\)X-rays. Since the mutation frequency did not reach the peak even at the highest dosage of EMS and PMS, more mutagenic effect may be expected from them by an appropriate treatment.

Variance on heading date of the M₂ plants, which fell within a normal range of the variation, tended to increase when the doses of each agent was increased. From the result of the maximum variance obtained, the mutagenic effect of each agent could be placed in the order, EI\(\gg\)EMS\(\gg\)PMS\(\gg\)X-rays. The correlation between the variance and the frequency of mutants was very high, when chemical agents were used ; the correlation coefficient was 0.985* for EI, 0.975* for EMS and 0.888* for PMS in contrast with a low value 0.347 for X-rays. These results strongly suggest that the mutagenic effect of chemical substances can be successfully estimated from the variance among normally appearing plants.

A further difference between chemical agents and X-rays was found in a relation of the frequency of mutant plants to the sterility of M₁ panicles. As shown in Fig. 2, the frequency of mutants depended much more upon the M₁ sterility
in chemical treatments than in X-ray irradiation. The frequency of mutants was increased by 0.17% with chemical agents and 0.02% with X-rays for every 1% increase of the sterility; the effect of four chemicals was in the order of EI (0.19%) > EMS (0.20%) > PMS (0.10%). The relation (EMS > PMS) confirms the result of Rao et al. (1965). Those findings seem to indicate that mechanisms of chromosomal aberration are different not only between chemical mutagens and ionizing radiations (Froese-Gertzen et al., 1964) but also among chemicals carrying similar radicals.

According to the further results obtained in the M₃ generation, no particular difference was found in genetic behaviors among mutants produced by different agents. Therefore, natures of those mutants can not be so much different each other.

Putting all the results together, it can be concluded that EI, EMS and PMS are more effective as a mutagen than X-rays if the treatment is given appropriately, and the frequency of mutants or the variance among normally appearing plants in the M₃ generation has a greater importance than the mutation spectrum as an index of the mutagenic effect of various mutagens on agronomic characters.

2. Effective Method of Treatment

Early determination of mutagenic effect is very important in hunting mutagens or finding their usefulness in breeding. In this regard, the relationship between mutational products and direct response of plants to treatment must be first investigated. Experiments were conducted in 1965 and 1966, in order to make clear this point.
Dry seeds of the variety, Gimbôzu, were treated in 1965 under different length of time and different temperature with various concentrations of eight kinds of alkylating substances, i.e., EI, EO, PO (propylene oxide), BO (butylene oxide), EMS, PMS, BMS (butyl methanesulfonate) and OMS (octyl methanesulfonate). BMS and OMS were used in the state of sol produced by mixing them with alcohol, xylene and polyethylene glycol, whereas the others were used as solution. In the \( M_2 \) generation, all the chemicals except BO and OMS induced chlorophyll mutations.

From the results mostly obtained by EI and EMS treatments, a particular tendency of dosage-response of chlorophyll mutation has been noticed, one of its examples is shown in Fig. 3. The dosage-response curve follows, in this case, to a cubic curve with two inflection points corresponding to the maximum and minimum values. This result suggests that there is non-linear response of mutation to chemical treatment.

![Fig. 3. An example of the dosage-response of chlorophyll mutation. The mutation rate is given by percent per \( M_1 \)-panicle progeny.](image)

Contrasting the dosage for the maximum point of mutation frequency to the degree of injury caused by that dosage, it was generally found that the maximum mutation rate was obtained by a dosage, which began to cause a remarkable reduction of germination rate of treated seeds, or which caused 30 to 50% reduction of the seed fertility in \( M_1 \). However, no conclusion is yet obtained about the most effective treatment in combination of various factors, such as concentration of chemicals, time of treatment and temperature.

In 1966, EI, EMS and PMS were used to treat dry seeds of the same variety, Gimbôzu. From the results of 1965, the following treatments were given; 1~4 hr., 0.05~1.0% concentration (v/v) for EI, 8~32 hr., 0.1~1.2% conc. for EMS,
Fig. 1. The dosage-response of the germination of treated seeds.
and 8~32 hr., 0.8~1.6% conc. for PMS, under three different temperatures 15°, 20° and 25°C.

Fig.4 shows the effect of different concentrations on the germination of treated seeds. Decrease of the germination rate was clearly observed when the level of all three factors, i.e., concentration, time and temperature were raised. With all chemicals, sigmoid curve was obtained for both concentration and time. In the case of temperature, however, the response curve became linear or parabolic for EMS and PMS, though its shape was not so clear for EI.

References


水稲におけるアルキル化物質の突然変異誘起効果と
効果的処理法について

山 県 弘 忠

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放射線にかわる mutagen として最近種々の化学物質が試用されるようになったが，それらの突然変異誘起に関する諸特性を解明するとともに，突然変異の誘起にもっとも効果的な処理方法を追究し，生物学的立場からより有効な変異誘起物質開発の端緒を探ることとは，育種上必然的な一要望事項であると考えられる。ここでは，筆者らが上記のような観点から，1963年以降水稲を用いて行なっている実験について，その概要を紹介する。

供試薬品は，各種のアルキル化物質 ethyleneimine (EI)，monoepoxide類 (BO, PO, BO)，diethylsulfate (DS)，および methanesulfonic acid ester 類 (MMS, EMS, PMS, BMS, OMS) であって，変異誘起処理は，これらの種々の濃度の溶液または懸濁液に水稻品種銀坊主の乾燥種子を浸漬することによって行なっている。
1. 突然変異誘起特性
1963年、EI、EO、DS、MMS、EMS および PMS（0.01～1.0％）を用いて処理を行なうとともに、比較のため乾燥種子にX線（5〜50kR）を照射した。処理条件は EI、EO および DS については 17〜18℃でそれぞれ 2.5、17 および 2 時間、MMS、EMS についてはいずれも、20〜21℃で17時間である。これらのうち EI、EMS、PMS および X線の各区については M_2 ならびに M_3 世代を育成し、主として出穂期に関する突然変異の出現状態を調べた。

M_2 では、各作区ごとに、原種に比べて約10日早生から約50日晩生に至る種々の出穂日変異体が生じた。それらの spectrum については作因間で特に異なる傾向は認められなかったが、出現率については、作因間で処理量に対する反応を異にし、最大出現率において EI>PMS>X線の関係があること、および M_1 基因性的低下に対する出現率増加の割合が化学物質とX線でかなり異なっていることなどが認められた。また M_2 において、出穂日に関して一応正常型と認定された個体の分散は、各作因とも処理量に伴って増大し、化学物質については変異体出現率との間に 0.8 以上の有意な正の相関が認められた。

各世代の結果を総合すると、出穂期変異のほか概して遺伝子変異に基づくと推定される実用形質の突然変異については、少なくとも EI、EMS および PMS は X線以上に効果的な変異誘起原であろうと推察される。

2. 効果的処理方法
1965年、EI、EO、PO、BO、EMS、PMS、BMS、および OMS を用い、EI および EMS をを中心に濃度のほか温度、時間に応じて種々の条件を設定して処理を行なった。その結果、BO および OMS を除くすべての物質において葉緑突然変異体の出現が認められたほか、EI および EMS において、葉緑突然変異体の出現率が処理量に対して特殊な、3 次数的増減を示す傾向がうかがわれた。

1966年には、EI、EMS および PMS を用い、前年度の成績に基づいて温度条件 3（15℃、20℃、25℃）、時間条件 4（EI、1〜4時間；EMS、PMS 8〜32時間）を設け、それぞれについて数種類の濃度（EI 0.05〜1.0％；EMS 0.1〜1.2％；PMS 0.8〜1.6％）の処理を行なった。その結果、処理種子の発芽率は、各物質とも、濃度、時間、温度の各要因に大きく支配され、濃度および時間に対してはそれぞれS字状反応を示すことなどが認められた。
INDUCTION OF MUTATIONS IN RICE
BY SOME CHEMICALS

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Studies on chemical mutagenesis in barley during the last decade showed that some chemicals induce mutations with frequencies two to three times higher than the highest frequencies obtained in radiation treatments (Ehrenberg et al., 1958; Heiner et al., 1960; Ehrenberg et al., 1961). In order to obtain knowledge on mutagenic effects of chemicals and to develop effective methods of treatments with chemicals in rice, experiments have been conducted since 1961 (Kawai and Sato, 1965; Sato and Kawai, 1965; Sato and Kawai, 1966). Results of the experiments obtained up to now are collectively presented in this paper.

Seeds of a rice variety Norin No. 8 were treated in all the experiments. Chemicals and treatment conditions were as follows:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration</th>
<th>Treatment time</th>
<th>Temperature</th>
<th>Water content of seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene imine (EI)</td>
<td>0.02~0.5%</td>
<td>1~5 hr</td>
<td>7°C, 30°C</td>
<td>12~13%</td>
</tr>
<tr>
<td>Ethylene oxide (EO)</td>
<td>0.1~0.6%</td>
<td>2~10 hr</td>
<td>30°C</td>
<td>12~13%</td>
</tr>
<tr>
<td>Diethyl sulfate (DS)</td>
<td>Saturated</td>
<td>1~20 hr</td>
<td>5 or 10°C, 30°C</td>
<td>6<del>7, 12</del>13% &amp; Soaked seed (12 hr)</td>
</tr>
<tr>
<td>Ethyl methane-sulfonate (EMS)</td>
<td>0.2~1.5%</td>
<td>3~24 hr</td>
<td>7°C, 30°C</td>
<td>6<del>7, 12</del>13%</td>
</tr>
</tbody>
</table>

X-ray treatment of dry and soaked seeds were also made for comparison between chemical and radiation effects.

(1) Effects on M₁ Plants

Effect of the treatments on M₁ seedling growth were naturally different depending upon the kind of chemicals and treatment condition. General idea could be obtained from Fig. 1.

In general, injuries in M₁ generation (expressed as growth reduction of M₁ seedling) increased with the increases of concentration, time of treatment and temperature, and with the decrease of water content of seed. In the case of DS treatment at 30°C, however, the increase of growth reduction with increasing times of treatment was not observed in treatments for six hours or more. Treatments
Fig. 1. $M_i$ seedling growth after treatments with three chemicals under different conditions. (Treatment condition is indicated as time of treatment (h)-temperature ($^\circ$C)-water content of seed (%). The same is in the following figures.)

with DS solution stored for six hours at 30°C after preparation had no influence on seedling growth. Konzak et al. (1961) have reported that half-life of DS in aqueous solution was 13.1 hours at 10°C and 1.01 hours at 30°C. The present results in DS treatment can be related to the half-life of this chemical.

Fig. 2. Relationship between $M_i$ seedling height and $M_i$ fertility after treatments with chemicals and X-ray.
Reduction of M₁ fertility was markedly different in treatments with different chemicals (Fig. 2). Fertilities of M₁ spikes, compared on the basis of growth reduction of M₁ seedlings, were in the following order of magnitude: EO>DS>EI>X-ray>EMS. Reduction of M₁ fertility in EMS treatment was to marked extent, however, these were very small in the case of treatments with EO and DS.

![Graphs showing relationship between concentration and number of chlorophyll mutations per 100 M₁ spikes for different chemicals.]

Fig. 3. Dose-mutation frequency relationships after treatments with three chemicals. (Treatment plots with tested M₁ spikes less than 50 were excluded. The same is in the following figures.)

(2) Chlorophyll Mutation in M₂

Frequency of chlorophyll mutations

Relationship between dosage and mutation frequency after treatments with three chemicals are presented in Fig. 3. Frequencies of chlorophyll mutations in treatments with doses lower than LD 50’s, generally, increased with increasing concentrations of chemicals. (The results on mutation frequencies in treatments with doses higher than LD 50’s were not so complete because of the small numbers of tested M₁ spikes).

In relation to growth reduction of M₁ seedling (Fig. 4), mutation frequencies were high in treatments which induced large growth reduction in the case of EI and EMS treatments. In the case of DS treatment, however, it was noticed that treatments at low temperature (5°C or 10°C) induced mutations, with relatively less M₁ injuries, at higher frequencies than those at high temperature (30°C). The results, as Nilan et al. (1964) stated in their experiments on barley, could be related to differential rates of hydrolysis reaction and diffusion of this chemical into biological system, which would result in a higher number of reactions in the spike.
Fig. 4. Influences of some factors on frequencies of chlorophyll mutations, compared on the basis of growth reduction of $M_1$ plants, after treatments with three chemicals.

Fig. 5. Comparison of frequencies of chlorophyll mutations on the basis of growth reduction of $M_1$ plants after treatments with chemicals and X-ray.
primordial tissues than in the external tissues.

The Number of chlorophyll mutations per M1 spike, when compared on the basis of M1 seedling height, was in the following order of magnitude: EI=EMS>DS>X-ray>EO (Fig. 5). The maximum number of chlorophyll mutations per 100 M1 spikes was 59 and 50 (although not shown in Fig. 5, because of the small numbers of tested M1 spikes) in EI and EMS treatments respectively, being four to five times higher than that (12) of X-ray treatment. The value in DS treatment was 17 (treatment of seed with water content of 12% for 12 hours at 10°C). This treatment reduced survival rate only by 35%. Higher frequencies of mutations could be expected in DS treatments with higher doses or appropriate methods of treatment.

Segregation ratio of chlorophyll mutations

Segregation ratio in M2 is considered to give some information on chimeric nature of M1 spike and also to be necessary for estimation of mutation rate (expressed as the number of mutations per initial cell or sector of M1 spike).

Segregation ratios (pooled value) in M2 after treatments with chemicals were different among them and, in general, low as compared with those in X-ray treat-

![Graph](image)

Fig. 6. Comparison of M2 segregation ratios of chlorophyll mutations after treatments with chemicals and X-ray.
ments. As a general tendency, they increased as doses increased. However, such a dose-dependence of $M_2$ segregation ratio was low in EO and DS treatments (Fig. 6).

Segregation ratios in $M_2$, compared on the basis of $M_1$ injury, were in the following order of magnitude: X-ray $>$ EMS $>$ EI $>$ DS $>$ EO. These results suggest that the number of sectors in $M_1$ spike decreases with the increase of dosage and that the number is larger in treatments with chemicals than with X-ray. It could not, however, be concluded that the relatively large number of sectors in $M_1$ spike was due to the delayed effect in the chemical treatments (Kawai and Sato, 1965).

The numbers of mutations per sector in $M_1$ spike in EI and EMS treatments were two to three times higher than that in X-ray treatment.

Spectrum of chlorophyll mutations

Since mutation spectra did not show any difference with the conditions in treatments with EO, DS, (EMS) and X-ray, comparisons were made among spectra which were obtained in treatments with different mutagens. Spectrum of chlorophyll mutations after EO treatment was not different from that of X-ray treatment.

Table 1. Chlorophyll mutation spectra after treatments with chemicals and X-ray

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Grouping by no. of mut. per 100 $M_1$ spikes</th>
<th>Total no. of mutations</th>
<th>Relative frequencies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Albina</td>
</tr>
<tr>
<td>EI</td>
<td>(a)~30.0</td>
<td>213</td>
<td>48.4</td>
</tr>
<tr>
<td>$\approx$</td>
<td>(b) 30.1~</td>
<td>322</td>
<td>31.4</td>
</tr>
<tr>
<td>EO</td>
<td></td>
<td>24</td>
<td>66.7</td>
</tr>
<tr>
<td>DS</td>
<td></td>
<td>545</td>
<td>44.2</td>
</tr>
<tr>
<td>EMS</td>
<td>(a)~30.0</td>
<td>383</td>
<td>41.8</td>
</tr>
<tr>
<td>$\approx$</td>
<td>(b) 30.1~</td>
<td>263</td>
<td>35.4</td>
</tr>
<tr>
<td>X-ray</td>
<td></td>
<td>424</td>
<td>50.5</td>
</tr>
</tbody>
</table>

Results of $\chi^2$ test

<table>
<thead>
<tr>
<th></th>
<th>EI(a)</th>
<th>EI(b)</th>
<th>EO</th>
<th>DS</th>
<th>EMS(a)</th>
<th>EMS(b)</th>
<th>X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI(a)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\approx$</td>
<td>***</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EO</td>
<td>N.S.</td>
<td>N.S.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>DS</td>
<td>*</td>
<td>**</td>
<td>N.S.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>EMS(a)</td>
<td>N.S.</td>
<td>*</td>
<td>N.S.</td>
<td>N.S.</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$\approx$</td>
<td>**</td>
<td>N.S.</td>
<td>N.S.</td>
<td>*</td>
<td>N.S.</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>X-ray</td>
<td>N.S.</td>
<td>***</td>
<td>N.S.</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>—</td>
</tr>
</tbody>
</table>

*: Significant at 5, 1, and 0.1% levels, respectively.
N.S.: Not significant.
Spectra after DS or EMS treatments were significantly different from that of X-ray treatment, that is \textit{albina} mutation being relatively less and \textit{viridis} and other mutations relatively more in DS and EMS treatments (Table 1).

It should be noted that, in the case of EI treatment, mutation spectra were significantly different in the treatments which induced mutations at different frequencies (Table 1). (In the comparison, mutation spectra were corrected for bias due to simultaneous inductions of two or more mutations of the same or similar phenotypes in an M$_1$ spike). Similar tendency was also observed in the case of EMS treatment, although the difference in spectra was not significant. Mutation spectrum in EI treatments which induced mutations at high frequencies (over 30.1 per 100 M$_1$ spikes) were significantly different from that of X-ray treatment.

Spectra after treatments with different chemicals were also significantly different from each other in some cases (Table 1).

(3) \textbf{Visible Mutation}

Studies on visible mutations have been carried out on materials from a part of the treatments. The results (up to M$_2$) in EI and EO treatments are presented in Table 2 and Fig. 7, together with those of treatments with X-ray (20 kR) or $\gamma$-ray (10-40 kR).

\textit{Frequency of visible mutations}

Frequencies of M$_2$ strains which segregated visible mutants were in the following order of magnitude when compared on the basis of M$_1$ seedling height: EI > X-ray > EO. The value in the treatment with 0.3\% EI solution for tow hours was 100\% (Table 2).

Similar relationship was observed on frequency of visible mutant types. (Mutant types are homozygous types for induced mutation(s), presumed from breeding behaviours of mutants and their phenotypes.) The number of mutant types per 100 M$_1$ spikes in treatment with 0.1\% EI solution was 95, being two times or more higher than that (42) of X-ray treatment. The highest number of mutant types per 100 M$_1$ spikes was 153 in treatment with 0.3\% EI solution. There seemed to be a parallel relation between the number of visible mutant types per M$_1$ spike and the number of chlorophyll mutations per M$_1$ spike, except the case of treatment with 0.3\% EI solution (Table 2).

The number of visible mutant types would not coincide with the number of visible mutations, and the former would be larger than the latter. Screening and classification of mutant types could not be carried out precisely after the treatments which induced mutations at high frequencies. The numbers of mutant types
Fig. 7 Variations of characters in mutant types induced by treatments with chemicals and radiations.
(Ordinate: Number of mutant types. Abscissa: Variation index, % of control)

in the table, particularly in treatments with high doses, would probably be underestimated ones.

Variations of characters in mutant types
Changes of characters in mutant types induced by chemicals and radiations were mostly towards lateness in heading, decrease in culm and panicle lengths,
Table 2. Mutation frequencies and $M_1$ injuries in treatments with chemicals and X-ray

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$M_1$ survival rate</th>
<th>$M_1$ seedl. height</th>
<th>No. of chlorophyll mut. per 100 $M_1$ spikes</th>
<th>Freq. of $M_1$ strains with visible mut. (%)</th>
<th>No. of visible $M_1$ mutant types per 100 $M_1$ spikes</th>
</tr>
</thead>
<tbody>
<tr>
<td>EI</td>
<td>0.1%</td>
<td>90</td>
<td>74</td>
<td>21.1</td>
<td>76.7</td>
</tr>
<tr>
<td>EI</td>
<td>0.3%</td>
<td>52</td>
<td>25</td>
<td>55.1</td>
<td>100.0</td>
</tr>
<tr>
<td>EO</td>
<td>0.3%</td>
<td>102</td>
<td>44</td>
<td>1.9</td>
<td>14.3</td>
</tr>
<tr>
<td>EO</td>
<td>0.5%</td>
<td>50</td>
<td>7</td>
<td>4.2</td>
<td>35.7</td>
</tr>
<tr>
<td>X-ray</td>
<td>20 kR</td>
<td>90</td>
<td>51</td>
<td>8.5</td>
<td>60.6</td>
</tr>
</tbody>
</table>

(1) Treatments of seed with water content of 12\textendash13%: 2hr-30°C treatment in chemicals.
(2) Variants in only fertility were excluded.

Grain size, panicle weight and number of spikelet per panicle. The number of panicles per plant was frequently changed to positive direction (Fig. 7).

When variations of characters in mutant types induced by EI were compared with those by $\gamma$-ray, though statistically significant difference was not observed in the number of panicles per plant, culm length, panicle length and 1000 grain weight, the significant difference was found in heading date, the number of spikelets per panicle, grain weight per panicle and seed set density. Early heading mutant types and mutant types with small panicles were relatively more frequent in mutant types induced by EI than those by $\gamma$-ray.

Proportion of mutant types with two or more mutations might be relatively high in mutant types after EI treatment which induced mutations at higher frequency than X-ray treatment. Mutant types originated from one and the same $M_1$ spike might carry the same mutation and the number of such mutant types would be different in treatments with EI and X-ray. When these are taken into consideration, further detailed studies are required to get a conclusion of difference in spectra which are obtained in treatments with the two mutagens.

The results in the present studies clearly show that EI and probably EMS are more powerful mutagens than radiations in inducing visible mutations in rice. They would also be more effective in practical mutation breeding, although further studies, especially on agronomic characteristics of induced mutations, are required to conclude this so definitely.

Acknowledgment: The author wishes to express his sincere thanks to Dr. T. Kawai for perusing this manuscript.
References


化学物質によるイネの突然変異の誘発

佐 藤 尚 雄
（農業技術研究所）

化学物質のイネに対する突然変異誘発効果および効果的な処理方法を明らかにするために1961年以降実験を行なっており、現在までに得られた結果の概要を述べる。

水稻農林8号の種子をEthylene imine（EI）、Ethylene oxide（EO）、Diethyl sulfate（DS）およびEthyl methanesulfonate（EMS）の溶液に、濃度、時間、温度、種子水分含量を変えて浸漬し、別に比較のため、気乾および浸水種子のX線処理を行なった。

1. 処理方法（M1）の障害

M1障害は、一般に、濃度、処理時間、温度が大きい程、また種子水分含量が低い程大きい。然し、DSによる高温下（30°C）での6時間以上の処理では、処理時間によるM1障害の増大は認められなかった。

M1総実率とM1生育障害との関係は化学物質によって著しく異なる。同じ生育障害でのM1総実率はEO>DS>EI>X線>EMSの関係にあり、EO、DS処理での総実率の低下は僅かであるが、EMS処理での低下は著しかった。

2. 業縁体突然変異（M2）
異なる条件での同じ化学物質による $M_1$ 糧当り突然変異数は、一般に、$M_1$ 障害の大きさ程大きいが、DS の低温処理では、高温処理に比較して、$M_1$ 障害が小さいにもかかわらず高い突然変異出現率が見られた。

$M_1$ 障害を基準にして比較すると、100 M 糧当り突然変異数は $EI ≃ EMS ≫ DS ≫ X線 ≫ EO$ の関係にあり、それぞれの処理での最高値は EI で 59、EMS で 50 で、いずれも X 線による値（12）の数倍に達した。DS 処理での最高値は17であったが、$M_1$ 障害の程度から見れば更に高い値を期待しうる。

同じ $M_1$ 障害で比較した $M_2$ 分離比 はおむろかに X 線 > EMS > EI > DS > EO の関係にある。また $M_2$ 分離比はドースの増加とともに増加する傾向が見られた。

葉緑体突然変異のスペクトラムは EO 処理と X 線処理の間には差が見られなかったが、DS および EMS と X 線との間には有意の差が認められた。EI 処理の場合には、高い率（100 $M_1$ 糧当り 30.1 以上）で突然変異を生ずる処理でのスペクトラムと低い率（100 $M_1$ 糧当り 30.0 以下）で突然変異を生ずる処理でのそれとの間に差があり、前者はX線によるそれとも有意に異なっていた。

3. 可視突然変異

EI および EO 処理後代での調査結果は次のようにある。

$M_1$ 障害を基準にして突然変異型の出現率を比較すれば EI > X線 > EO の関係にあり、EI の 0.1％ 溶液処理での 100 $M_1$ 糧当り数は95でX線処理（20 kR）による42の約2.5倍であった。また最高の値は EI の 0.3％ 溶液処理での 153 であった。

可視突然変異型の出現率は葉緑体突然変異出現率とはほぼ平行的な関係を示すものと見られる。

可視突然変異型の形質変異では根生、短縮、小粒化したものが多く、雛数では増加程度への変化がかなり見られた。EI と 7 線の比較では、雛数、稈長、雛長、1000 粒重では差が見られなかったが、出穂日、一穂粒数、一穂重、着粒密度では有意な差が見られ、EI で早生化または小穂化した変異型が多かった。突然変異型は必ずしも個々の突然変異に対応するとは限らず、スペクトラムが異なるか否かは更に検討を要する。
A RESULT OF ETHYLENE IMINE TREATMENT IN MALTING BARLEY

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Kihara Institute for Biological Research, Yokohama, Japan

Dry seed of a two-rowed malting barley strain Ko-A was treated with ethylene imine solution, and the rate and kinds of mutation were investigated. Other seeds were treated with gamma-rays to compare the effect of the two mutagens. Table 1 shows the results obtained in the M₁ generation. Three spikes of each M₁ plant were bagged and the one with the highest number of seed was picked to be carried into M₂. Throughout their development, M₂ plants were compared with non-treated controls, and plants which showed apparent variation were selected as possible mutants. The frequency of variant plants per M₂ strain was higher after the stronger treatments had been done (Table 2).

As shown in Table 3, the rate of mutation affecting chlorophyll expression in M₂ plants was much higher when treated with ethylene imine, while in the gamma-ray plot the frequency of sterile or poorly fertile plants was high.

It was observed that the short-culm character often appeared together with other variations in the same plant. In particular, short culm was associated with complete male sterility, early maturity and dense spike. Those plants, which had short culms apparently resulted from early maturity, were classified as early maturity mutants, and those plants which had both short culm and dense spike

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of treated seed sown</th>
<th>No. of seedlings obtained (%)</th>
<th>No. of M₁ plant matured (%)</th>
<th>Seed fertility (%) bagged</th>
<th>open</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene imine 0.4% 1 hour</td>
<td>200</td>
<td>174 (87.0)</td>
<td>152 (76.0)</td>
<td>67.40</td>
<td>83.17</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 2 hours</td>
<td>200</td>
<td>173 (86.5)</td>
<td>161 (80.5)</td>
<td>86.51</td>
<td>88.07</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 1 hour</td>
<td>200</td>
<td>178 (89.0)</td>
<td>159 (79.5)</td>
<td>81.13</td>
<td>91.21</td>
</tr>
<tr>
<td>γ-ray 15kR I</td>
<td>200</td>
<td>178 (89.0)</td>
<td>150 (75.0)</td>
<td>60.83</td>
<td>80.43</td>
</tr>
<tr>
<td>γ-ray 15kR II</td>
<td>1,100</td>
<td>1,001 (91.0)</td>
<td>929 (84.5)</td>
<td>68.75</td>
<td>80.97</td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>188 (94.0)</td>
<td>169 (84.5)</td>
<td>77.45</td>
<td>92.70</td>
</tr>
</tbody>
</table>
Table 2. Number of $M_2$ families which segregated possible mutant plant

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of $M_1$ spikes sown</th>
<th>No. of $M_2$ families investigated</th>
<th>No. and percent of $M_2$ families which showed variation (% of $M_2$ strain investigated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene imine 0.4% 1 hour</td>
<td>150</td>
<td>142</td>
<td>37 (26.1)</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 2 hours</td>
<td>150</td>
<td>144</td>
<td>38 (26.4)</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 1 hour</td>
<td>129</td>
<td>127</td>
<td>17 (13.4)</td>
</tr>
<tr>
<td>7-ray 15kR</td>
<td>149</td>
<td>139</td>
<td>24 (17.3)</td>
</tr>
<tr>
<td>Control</td>
<td>60</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>638</td>
<td>612</td>
<td>116</td>
</tr>
</tbody>
</table>

Table 3. Kinds of mutation and their frequency, as confirmed in $M_3$

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Kind of mutation generation</th>
<th>Chlorophyll expression</th>
<th>Plant morphology</th>
<th>Fertility</th>
<th>Heading time</th>
<th>Calm length</th>
<th>Spike length</th>
<th>Total mutations</th>
<th>No. of $M_2$ families involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethylene imine 0.4% 1 hour</td>
<td>$M_3$</td>
<td>19</td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>44</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>No. picked up in $M_2$ as possible mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>8</td>
<td>7</td>
<td>-</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of mutations confirmed (% of No. tested)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(37.5)</td>
<td>(87.5)</td>
<td>-</td>
<td>-</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total no. of mutations (% of total mutations)</td>
<td>(51.4)</td>
<td>(8.1)</td>
<td>(13.5)</td>
<td>(16.2)</td>
<td>(0.0)</td>
<td>-</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 2 hours</td>
<td>$M_3$</td>
<td>16</td>
<td>5</td>
<td>12</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>46</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>No. picked up in $M_2$ as possible mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>3</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of mutations confirmed (% of No. tested)</td>
<td>(100.0)</td>
<td>(11.1)</td>
<td>(100.0)</td>
<td>(100.0)</td>
<td>(100.0)</td>
<td>-</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total no. of mutations (% of total mutations)</td>
<td>(42.1)</td>
<td>(13.2)</td>
<td>(31.6)</td>
<td>(2.6)</td>
<td>(2.6)</td>
<td>(7.9)</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 1 hour</td>
<td>$M_3$</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>No. picked up in $M_2$ as possible mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of mutations confirmed (% of No. tested)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(100.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total no. of mutations (% of total mutations)</td>
<td>(60.0)</td>
<td>(0.0)</td>
<td>(20.0)</td>
<td>(0.0)</td>
<td>(20.0)</td>
<td>-</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>7-ray 15kR</td>
<td>$M_3$</td>
<td>3</td>
<td>1</td>
<td>12</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>No. picked up in $M_2$ as possible mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>No. tested</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No. of mutations confirmed (% of No. tested)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(0.0)</td>
<td>(50.0)</td>
<td>-</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total no. of mutations (% of total mutations)</td>
<td>(15.0)</td>
<td>(5.0)</td>
<td>(60.0)</td>
<td>(5.0)</td>
<td>(15.0)</td>
<td>(0.0)</td>
<td>20</td>
<td>16</td>
</tr>
</tbody>
</table>
were classified as short-culm mutations (Table 3). As for variation in fertility, besides plants which showed about 50% of seed setting, complete male-sterile plants were found. It is considered that the former may be due to chromosome aberration and the latter to gene mutation. When they had short culm, complete male steriles were classified as fertility mutants. In morphological variation, dwarfness and liguleless were included.

Although it was thought that the appearance of combinations of two characters could mostly be attributed to expression of a single pleiotropic gene mutation rather than to two independent mutations, this had to be tested. Moreover, since the characters observed may vary due to environmental effects, it was necessary to confirm the mutational character of the variant plants. The results of progeny test in M₃ generation showed that the number of real mutations affecting heading and time of maturity was very much smaller than that appeared in M₂ (Tables 3 and 4). Thus it is difficult to select the mutants which have difference in maturity from the parent strain by a few days: most of plants which were thought to be a few days earlier in maturity were a result of environmental variation. It was confirmed that sixty percent of culm-length variants and all of the spike-length variants were due to mutation. The real number of mutations, corrected after M₃ progeny tests, is shown in Table 3, and the number of mutations per M₁ spike is summarized in Table 5. The highest number of mutations from one M₁ spike

---

Table 4. Rate of confirmation of mutation of variant plants picked up in M₂

<table>
<thead>
<tr>
<th>Character</th>
<th>No. picked up in M₂</th>
<th>No. tested</th>
<th>No. of mutations confirmed</th>
<th>Percentage of no. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heading time</td>
<td>36</td>
<td>34</td>
<td>4</td>
<td>11.8</td>
</tr>
<tr>
<td>Culm length</td>
<td>16</td>
<td>15</td>
<td>9</td>
<td>60.0</td>
</tr>
<tr>
<td>Spike length</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 5. Frequency of mutation per M₁ spike

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of M₂ families investigated</th>
<th>No. of mutations per M₁ spike</th>
<th>Total mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Ethylene imine 0.4% 1 hour</td>
<td>142</td>
<td>110</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(77.5)</td>
<td>(19.0)</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 2 hours</td>
<td>144</td>
<td>111</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(77.1)</td>
<td>(19.4)</td>
</tr>
<tr>
<td>Ethylene imine 0.2% 1 hour</td>
<td>127</td>
<td>122</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(96.1)</td>
<td>(3.9)</td>
</tr>
<tr>
<td>7-ray 15kR</td>
<td>139</td>
<td>123</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(88.5)</td>
<td>(9.4)</td>
</tr>
</tbody>
</table>
was three.
Among 86 mutations obtained, three short-culm mutants were thought to be interesting in further investigation for breeding purposes.

ビールムギの育種における化学物質による突然変異

村 松 幹 夫
(本原生物学研究所)

ビールムギの1品種交Aの乾燥種子を用いてEthylene imine処理および7-線照射を行なって突然変異の頻度および種類を調査した。とくに育種上重要と思われる形質に重点をおいて比較検討した。処理条件と処理第1代植物(M₁)の栽培結果を第1表に示す。M₁植物は1株より3種つづ袋掛けを行なって採種した。そのうち最高の着粒を示す1種を選んでM₂の1系統とした。

M₂における変異個体の調査は生育全期間にわたって行い、対照と比較して明らかに変異を示している個体を選抜した。変異植物の出現頻度は処理濃度が高い区において高い（第2表）。変異を形質別に分類し比較すると、Ethylene imine処理では7-線に比べて葉緑素の変異の頻度が非常に高く、一方7-線では稔性変異が高い（第3表）。また2種類の変異が同一個体に伴って出現する場合が比較的多い。とくに短稈変異はしばしば不稔、早生および密穂を伴っている。第3表では早生のため短稈である変異は出穂期変異とし、短稈で密穂の変異は穂長変異に入れた。稔性変異には雄性不稔のほか約50%の着粒を示すものがあり、明らかに前者は遺伝子突然変異、後者は染色体異常のために考えられる。雄性不稔が短稈変異を伴っている場合も稔性変異とした。形態変異としては矮性や葉舌欠如などが出現した。同一個体に出現した2種類以上の変異は同一遺伝子の多発現現による場合と、二種の突然変異による場合が考えられる。また環境による変異が含まれる可能性が高い。

したがって出穂期、穂長および稔長変異についてM₃世代を栽培して突然変異の確実性を調査した（第3表）。その結果M₂で出穂期変異として選抜されたもののうち、実際の突然変異によるものは非常に低く、11.8%であったが、一方穂長変異では60%，稔長変異では100%であった（第4表）。また第5表に示すようにM₁の1種あたりについて最高3種の突然変異が確認された。

全突然変異系統を通じて、育種上さらに世代を進めて調査する価値があると判定されたのは3系統であった。
パネル討論

山口大曾根氏は、1）EIと電離放射線とでは、誘発突然変異の種類が異なること、2）各mutagenによって生成した突然変異にはいずれも多面的な形質発現がみられるること、3）EIでは、薬剤突然変異に伴ってほかの突然変異がおきていることを明らかにしました。

また、山口氏は、1）化学物質処理の場合、濃度の増加につれて突然変異率も高まる傾向にあること、2）化学物質の種類によって、突然変異頻度の最高峰が異なり、その順序はEI＞EMS＞PMS＞X線であること、3）処理方法が重要であり、薬品を処理した種子の発芽率が少し低下するのは濃度が高すぎている突然変異頻度が認められるということを述べられました。

また、佐藤氏は、1）用いる化学物質の種類によっては、処理方法を十分に検討しないと、2）X線よりも高い突然変異頻度を示す化学物質が多いこと、3）化学物質で得られる突然変異体の分離比は、X線のそれよりも低いこと、4）低い突然変異が生じる例はEI処理で突然変異のスペクトルは、X線の場合と差がないが、高い突然変異を生じる処理では、突然変異スペクトルがX線の場合と異なっていることを報告されました。

最後に、小西氏はEMS処理によってオオムギで得られた突然変異体についてスライドを中心にして説明されましたが、1）EMS処理次代で、雑交系統にしてまとめて三種の突然変異型がみられること、2）得られた突然変異には優性変化のものもあること、3）処理当時に表型描写も出現すること、4）今までに記載のないような珍しい突然変異も生ずることに要約されました。

私たちの研究室では、化学物質の使用によって高等植物の染色体の微細構造を解明し、その結果として有効な育種方法をつくり上げようとして実験を行なっています。現在までに得られました1, 2の知見をのべ、パネル討論の話題にしていただくために私の考えを出すことにします。

化学物質の処理時間を決める場合には薬剤の分解を考えてみる必要があり、一定濃度で処理時間を変えるときは薬剤そのものがいかんのか、またはその分解生成物がいかんかどうかが問題であります。このときは各薬剤について分解の反応速度定数（解離定数）を知る必要があるのではないかと思います。また、EIでもDESでも得られた突然変異体の分離比が低く、濃度をすますと分離比が増加します。この分離比が低い場合には突然変異モザイクや遅延突然変異の関与が考えられます。ここで突然変異がDNAの塩基変化でおこると仮定しますと、分離比が低いほうはうまく説明できますが、これを証明するためには塩基アナログによる突然変異誘発実験が必要でしょう。また化学物質の場合、X線よりも分離比が低いことは突然変異体発見の確率を小さくし、不利になりますから、化学物質の突然変異頻度はX線の突然変異頻度よりもかなり高くなければ使用できていないでしょう。化学物質処理で濃度を高きますと2コ以上の形質が変化した突然変異がかなり多くなるということが
あれば、使用目的に応じて、すなわち突然変異を直接利用する場合には処理濃度を低くし、また交配母本として使用する場合には突然変異の種類が多い方がよいので処理濃度は高くすることが必要ではないかと考えております。

ただいまより、パネル討論に入りますが、話を提供された三氏に、補足すべき点がありましたら教えていただきます。

大倉根 他の二氏の報告と比べた場合、共通のなものとして、1）M1の異常事象は電離放射線の方が大きいこと、2）M1の塩化水素酸性変異は、EIなどで高いこと、3）塩化水素酸性変異のスペクトルの中で viridis が多いこと、4）M2での転座型の部分不稔はX線の方が多いこと、5）EIでは早生が出やすいこと、6）突然変異に多面的な形質発現がみられることがあげられますし、他方、共通でないものとして、1）EIは塩化水素酸性変異頻度を高くするが、上場で発現される突然変異の頻度も同時に高いという点。2）高濃度（0.7％）のEI処理での分離比はX線の場合とほぼ同じであった。しかし、処理濃度の増加と共に、分離比が高まるところを、佐藤氏の結果と同様であるのではない。

山根 これは化学物質の濃度を容量パーセントで表していますが、他の二人の方はどのように表示方法をとっていますか。

大倉根 重量パーセントで表しています。

佐藤 私も同じように重量濃度を使っています。ここで私の報告について一つ補足しませんか。

松尾 山県氏によれば、化学物質処理の場合、M1の異常事象の減少に平行して突然変異率が増えることでしたし、これは濃度と処理に対する平均値についていえるのか、あるいは同じ処理の中の個体の変異性——変異性の個体変異があるとすれば——に応じているのか、どちらですか。

山県 X線に比べて化学物質は塩化水素酸と突然変異率とに強い関連性をもっていることを述べた際には、処理区の平均値で表しました。一方、各処理区内の個体変異と突然変異率との関係をみますと、たとえば不稔率80％程度の飼で最高の突然変異率が得られました。藤井 電離放射線と化学物質の育種的利用について比較すると、前者的場合にはLETと突然変異率の関係や健全度と突然変異率の関係などの基礎的な研究データがかなり多くつかみ上げられているのに反し、後者は処理濃度、処理時間と突然変異率の関係などの基礎的知識が不足している現状ですから、私はまだまだ放射線の方が育種上有効な手段だと思って
います。
山口 では、化学物質の濃度と突然変異率の間には直線的な比例関係があると結論してよいか、またどの化学物質が mutagen として有効かという問題を中心に討論を進めていきたいと思います。
山隈 私の実験では突然変異率が濃度の增加につれて一旦上昇して最高値に達し、それから下って再び上昇するような例もありましたので、両者の間に必ずしも比例関係があるとは断言できません。
佐藤 DES 処理の場合には過飽和溶液を使用しているので濃度がはっきりしていませんし、また一定濃度で処理時間を変えたときには加水分解の問題がありますので、比例関係をいうことができません。EMS 処理の場合には、大体その関係が成り立つようです。また EI 処理についても、実験例は少ないのですが、比例関係が存在しているようです。
仲尾 この点は非常にむずかしい問題です。たとえばナイトロジェン・マスタードは化学的にはエチルイミダゾール・イオンになって働きますが、そのイオンの持続時間も問題になります。また解離恒数は純水中と、種子や動物体内からの浸出物質がある場合とは異なってきます。したがって個々の化学物質とあつまう材料によっていちいち違ってきまですから、一般的な結論は出せないと思います。
山隈 山口氏によれば、エチル化物質に比べてメチル化水は高い毒性をもち、DNA 分子に致命的に働かないのでです。これに関連した点として、もし側鎖の長さを大きくした場合、それにともなう溶解度の減少や処理時間等の問題があり、これが有効に働くかどうかをきめるのは難しいようです。
大曾根 化学物質では加水分解によって突然変異率がプラトーに達するということのほかに、X 線の飽和効果には不確定性が大きく原因するということがありますので、EMS や EI でも M1 の不確定性が突然変異率のプラトーに関係しているのではないかと思われます。
私たちの実験では、EI の 0.7% 処理と X 線の 20kR 処理がほぼ同の生存率を示しましたので、両者の突然変異について比較をおこないましたが、もう少し低い濃度を用いたときにも同様な関係が見られるかどうかは不明です。
山口 Gaul 氏はオオムギで、穂絹系統法ではなくて、次代の播種個体あたりの突然変異体数で突然変異率を算出しますと、EMS の濃度と突然変異率の間に直線関係があると報告しています。このように突然変異率の表わし方によって比例関係がでてくる場合もありますから、イネでも再検討の必要があると思われます。
つぎに化学物質の処理濃度を高くすると多面的な形質発現を示す突然変異体が多くなるかどうかという点について、ご意見をおいただきたい。
佐藤 EI の 0.3% 処理では、1 粒粒数のような量的形質に近い形質が非常に低い値を示すようになりました。したがって確証はしないのですが、多数の遺伝子が変っているものと思われました。おそらく非常に高い濃度で得られた育種的に好ましくない変異体は、二つ以上の突然変異を含んでいる場合が多いのではないかと考えています。
山口 この問題は突然変異育種法に関係する大切な問題だと思います。たとえば、前に討論した処理濃度と突然変異率の関係に関連していますが、濃度を高めた場合に何種類もの
突然変異が同時におこるということはあとの育種操作を相当むずかしくします。処理濃度と突然変異の多面的な形質発現に比例関係があるとすれば、突然変異体を直接的に育種に利用する場合には、低い濃度で処理することが必要ではないでしょうか。この問題はこれからも研究すべき課題かと思います。

電離放射線と化学物質により誘発される突然変異型の差については、後者ではいわゆる遺伝子突然変異が多くいわれていますが、天野氏からトウモロコシの実験結果を報告していただきます。

天野　私は化学物質として主に EMS を使用しました。実験材料はトウモロコシで、第 9染色体上に挿入やすい遺伝子が三つ（I, Sh, Wx）並んでいる系統を用いました。放射線としては LET の高い、つまり破壊力の大きい 253U よりの分裂放出中性子を使い、その効果を EMS と比較しました。両者とも種子を処理しました。処理後成熟までに "Selection" が働いたためか、両者とも得られた突然変異は三つの遺伝子のうちの一つが変化した。一見 point mutation といわれるものが多かった。EMS では一つの遺伝子座あたり 23～27の突然変異が得られたのにに対し、中性子ではわずか 4～6 の効率でありました。その後この種の実験を合計 4 回おこないましたが、染色体の動原体に近いもの、つまり染色体の内側のものは、放射線の場合突然変異が起こりにくいような傾向にありました。これに対し EMS 処理をおこなった場合、染色体の外側にあてて種皮の色を支配する遺伝子と内側にあててモチ・ウルチを決定する遺伝子について比較すると、4 回の実験とも、後者の突然変異率がやや高く現われてきました。したがって、突然変異スペクトルは放射線と EMS で異なっていると断言してもよいのではないかと思われます。

さらにモチ・ウルチの場合も、花粉で解析できるという特性を利用して、EMS の効果を調べたところ、突然変異は waxy つまりモチ遺伝子の、ある広さを持ったスロットンの中に、ほぼ均等に分布しているような結果が得られました。また放射線の場合には、染色体の再結合が非常に起こり難く、生存率も低くなっていた。他方、EMS では生存率がさほど落ちていません。つまり連鎖している他的標識遺伝子の分離比を見ると、EMS でははっきりと 3：1 になります。

したがって高い生存率のもとで単一遺伝子の突然変異を狙う場合には、放射線よりも化学物質の方が可能性が高いと考えます。もっとも化学物質の場合には、モチとウルチの中間段階にあつゆる "leaky" な突然変異が放射線よりも現れやすいという傾向もあるようですが。

山口　化学物質で処理された場合には、不稔をおこす突然変異の多いことを Gaul 氏は報告しています。イネにおいても、育種的利用の面で、この傾向があるかどうかを確めてみるべきでしょう。

西村　私は主としてチェリップを材料にしておりますが、生長点が球根の中に入っているので、化学物質を使うことは技術的に困難です。こういうものに対する容易な処理法も開発・工夫していただきたい。

山口　予定時間も過ぎましたから、討論はここで打切ることにします。化学物質による突然変異誘発の研究を発展させるためには、今後も更に多くの基礎的データを積み重ねていかねばならないと考えています。
RELATIVE RADIOSENSITIVITIES OF THREE DEVELOPMENTAL STAGES, i.e., FLORAL DEVELOPMENT, GAMETOGENESIS AND EMBRYOGENESIS UNDER CHRONIC GAMMA IRRADIATION

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Many characters, such as 100 per cent lethality, severe growth inhibition and slight growth inhibition of vegetative growth, have been adopted as the criteria of plant radiosensitivity. However, from the viewpoint of plant breeding by radiation, seed productivity can be considered as the best criterion in seed propagated species, because seed productivity is essential character for plant breeding in these species. Relatively few reports are available describing such effects (Gomez Campo and Martiner, 1963; Mikaelson and Aastveit, 1957; Nybom, 1956; Nybom et al., 1956; Sparrow and Singleton, 1953; Woodwell and Oosting, 1965). Different

Fig. 1. Regression of daily exposure required to reduce seed set by 50 per cent plotted against interphase chromosome volume for 20 herbaceous diploid species. (Data from Table 1). (Yamakawa and Sparrow 1965)
Table 1. The relationship between interphase chromosome volume and daily exposure necessary to reduce seed set by 50 per cent in 20 herbaceous diploid species

<table>
<thead>
<tr>
<th>Plant number</th>
<th>Species and somatic chromosome number (2n)</th>
<th>Interphase chromosome volume ($\mu^3\pm$ S. E.)</th>
<th>50% seed setting (R/20hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Allium cepa</em>(16)</td>
<td>38.2±1.72</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td><em>Allium fistulosum</em>(16)</td>
<td>29.5±1.58</td>
<td>23</td>
</tr>
<tr>
<td>3*</td>
<td><em>Hordeum vulgare</em>(14)</td>
<td>18.8±0.78</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td><em>Pisum sativum</em>(14)</td>
<td>15.1±0.84</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td><em>Spinacia oleracea</em>(12)</td>
<td>10.8±0.46</td>
<td>90</td>
</tr>
<tr>
<td>6*</td>
<td><em>Phaseolus vulgaris</em>(22)</td>
<td>8.7±0.42</td>
<td>55</td>
</tr>
<tr>
<td>7</td>
<td><em>Solanum melongena</em>(24)</td>
<td>8.0±0.35</td>
<td>80</td>
</tr>
<tr>
<td>8*</td>
<td><em>Phaseolus vulgaris</em>(22)</td>
<td>7.6±0.26</td>
<td>75</td>
</tr>
<tr>
<td>9</td>
<td><em>Lycopersicum esculentum</em>(24)</td>
<td>7.4±0.34</td>
<td>85</td>
</tr>
<tr>
<td>10</td>
<td><em>Apium graveolens</em>(22)</td>
<td>7.2±0.35</td>
<td>50</td>
</tr>
<tr>
<td>11</td>
<td><em>Cucumis sativus</em>(14)</td>
<td>6.5±0.19</td>
<td>240</td>
</tr>
<tr>
<td>12</td>
<td><em>Capsicum frutescens</em>(24)</td>
<td>6.5±0.34</td>
<td>110</td>
</tr>
<tr>
<td>13</td>
<td><em>Cucumis melo makawa</em>(24)</td>
<td>6.3±0.19</td>
<td>270</td>
</tr>
<tr>
<td>14</td>
<td><em>Beta vulgaris cicla</em>(18)</td>
<td>6.2±0.15</td>
<td>130</td>
</tr>
<tr>
<td>15</td>
<td><em>Brassica oleracea capitata</em>(18)</td>
<td>5.8±0.30 (50)†140‡‡</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td><em>Daucus carota sativa</em>(18)</td>
<td>5.8±0.23 (45)†150‡‡</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><em>Citrullus vulgaris dulce</em>(22)</td>
<td>5.3±0.25</td>
<td>170</td>
</tr>
<tr>
<td>18</td>
<td><em>Raphanus sativus</em>(18)</td>
<td>5.2±0.18 (45)†150‡‡</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td><em>Brassica pekinensis</em>(20)</td>
<td>4.3±0.12 (60)†180‡‡</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td><em>Cucurbita maxima</em>(20)</td>
<td>3.7±0.14</td>
<td>280</td>
</tr>
<tr>
<td>21*</td>
<td><em>Oryza sativa</em>(24)</td>
<td>3.2±0.18</td>
<td>250</td>
</tr>
</tbody>
</table>

* Seed set data provided by A. Yamashita for *Hordeum vulgare*, by Y. Takagi for *Phaseolus vulgaris* and by S. Tanaka for *Oryza sativa* at the Institute of Radiation Breeding (Omiya, Ibaraki, Japan).
† Values for main stem and primary branch.
‡‡ Values for secondary and tertiary branch.
(Yamakawa and Sparrow, 1965)

Radiosensitivity among plant species judged by seed productivity was investigated by Yamakawa and Sparrow (1965), where the radiosensitivities of 21 cultivated herbaceous plants have been determined by daily exposure causing fifty per cent viable seed set following chronic exposure through all reproductive stages from flower bud differentiation to seed maturity. The exposure rates reducing seed productivity to 50 per cent of the unirradiated controls ranged from 18 to 280 R/20 hr day, while interphase chromosome volumes (ICVs) ranged inversely from 38.2 to 3.2$\mu^3$(Table 1). The results have a clear inverse relationship between ICV and exposure rate required to reduce yield of viable seed by 50 per cent (Fig. 1). This inverse relationship was in agreement with earlier results by Sparrow.
Fig. 2. Regressions comparing the daily exposure required to produce various endpoints as plotted against interphase chromosome volume for various herbaceous species. The data for lethality, severe growth inhibition and slight growth inhibition were obtained by Sparrow (1965) and Sparrow et al. (1965). The data for 50 per cent seed set are from Table 1. (Yamakawa and Sparrow 1965)

and others which showed a similar relationship between ICV and other radiobiological end points (Sparrow, 1962, ’64, ’65; Sparrow et al., 1961; Sparrow and Evans, 1961; Sparrow and Miksche, 1961; Sparrow et al., 1965; Sparrow and Woodwell, 1962) (Fig. 2). A comparison of the regressions for 50 per cent viable seed production and slight growth inhibition showed that approximately one-half the daily exposure required to produce a slight effect on growth was sufficient to reduce viable seed production by 50 per cent, indicating that the reproductive stages are much more sensitive than the vegetative stages. However, the reason for the greater sensitivity of the reproductive stages was not clear.

The purpose of the present paper is to investigate the different radiosensitivity among developmental stages. There are many papers reporting that the reproductive stages are much more sensitive than the vegetative stages (Kawai,
1962; Mikaelson and Aastveit, 1957; Nybom, 1956; Nybom et al., 1956; Sparrow and Christensen, 1953; Sparrow et al., 1961; Sparrow and Pond, 1956; Sparrow and Woodwell, 1962). However, most of the experiments on stage sensitivity of higher plants were concentrated to the radiosensitivity of microsporogenesis at cellular level. There was as much as 50-fold difference in sensitivity among the various stages in microsporogenesis of *Trillium erectum* (Sparrow et al., 1952). In *Tradescantia*, it was found that chronic exposure to ionizing radiation induce more chromosome aberrations in cells undergoing meiotic and post meiotic divisions than those in premeiotic somatic division (Sparrow and Pond, 1956). Proembryonic stage was also shown as one of the radiosensitive stages with barley (Mericle and Mericle, 1957). Flower bud development is also one of the important developmental stages of higher plants, and has well been known to be very sensitive to the environmental conditions such as temperature and light. Nevertheless, there are few reports, if any, discussing the radiosensitivity of flower bud developmental stage. Thus, relatively few reports are available in the stage sensitivity judged by seed productivity under chronic exposure, in spite of its importance for finding out the most effective irradiation method to get the maximum mutation yield.

The present experiments were carried out at the Institute of Radiation Breeding, Ministry of Agriculture and Forestry, Ibaraki, Japan, from 1962 to 1964, at Brookhaven National Laboratory of U.S.A. from 1964 to 1965, and again at the Institute of Radiation Breeding in 1966.

I Three Radiosensitive Stages Found in Tomato Plants

It seems that the greater radiosensitivity of the reproductive stages may result from: (a) greater nuclear volume of meiotic prophase stages, (b) longer period of irradiation because meiosis usually lasts longer than mitosis, (c) the hazard posed by genetically deficient nuclei passing through the haploid stages, or (d) unusually high sensitivity of the developmental stages involved in the formation of the flower and/or seed (i.e. the formation of floral organs, the zygote or the proembryo). Further experiments were required to determine the developmental stages having the high radiosensivities.

1. Materials and Methods

*Lyopersicum esculentum*, horticultural variety “Shugyoku”, was selected because the plants can produce fresh flowers successively over a long period of time.

Method of determining the effective stages to reduce seed set. The plants were exposed to 280, 500 and 1000 R/day for 5 days, when the flowers of the second
flower cluster were in bloom. The first flower of the first flower cluster was about 10 days after anthesis and the flowers of the fourth flower clusters were about 25 to 30 days before anthesis at the time of exposure. Flowering dates and flower position (for example, the 2nd flower of the 3rd flower cluster) were checked and scored for all flowers, and viable seeds per fruit were counted when they matured.

Method of determining the beginning of effective stages to reduce viable seed set. The plants were exposed to 57, 75, 120 and 190 R/day chronically from just before anthesis of the first flower cluster up to seed maturation. The plants possessed flowers of various developmental stages. Flowers of the fourth and fifth flower clusters were before flower bud initiation, and the flowers of the first flower cluster were completely differentiated at the beginning of exposure. Flowering dates, flower positions and viable seeds per fruit were scored or counted in the same way as abovementioned.

Observations of flower bud initiation and meiotic division. In this experiment only two developmental stages were checked. The time of flower bud initiation was determined by observing the growing points under dissecting microscope, and the time of meiotic division was determined by smearing the anthers in buds of different size. A more detailed discussion of flower development of tomato plants were given in section II.

Fig. 3. Seed set of *Lycopersicum esculentum* exposed to 280 (open circle), 500 (double circle) and 1000 R/day (closed circle) for five days at different reproductive stages. The scoring results are placed over the midpoints of each five days exposure period. The dashed line is the result of a later experiment being done for further test of zygotic sensitivity.
2. Results and Discussion

*Sensitive stages to reduce viable seed set.* After 5 days exposure to 280, 500 and 1000 R/day, the number of viable seeds per fruit was determined for each flower from the first to the fourth flower cluster when they matured (Fig. 3). The result showed that the viable seed set was reduced at three stages. They were about 25 and 10 days before anthesis and about 10 days after anthesis. The

![Graph showing effects of different doses of radiation on seed set.](image)

Fig. 4. Seed set of tomato plants (*Lycopersicum esculentum* H.V. Shugyoku) chronically exposed from different developmental stage to seed maturation. Note that exposure from prefloral developmental stages does not further reduce seed set beyond the reduction effected by irradiation of flower developmental stages. (Yamakawa and Sparrow, 1966)
observation of growing points under dissecting microscope showed that flower bud
initiation occurred about 25 days before anthesis, and smearing of anthers showed
that meiotic division of pollen mother cells took place 10 to 8 days before anthesis.
Smith (1935) has given detailed description of the embryo and its development of
_Lycopersicum_, and reported that the first two-celled embryos were found 94 hrs
after pollination, and the dermatogen, the periblum, the periblum initials, and
the plerome differentiated 224 hrs after pollination. Although it is difficult to
determine the exact stage of embryos at the time 10 days after anthesis which
was shown to be effective to reduce seed set in this experiment, it can be said
from Smith's experiment that this effective stage is a certain early stage of
embryogenesis.

The results of seed count for fruits of plants which were irradiated chronically
from just before anthesis of the first flower clusters to seed maturation of the
fifth flower clusters was shown in Fig. 4. The result showed that seed set per
fruit depended upon the length of exposure when once the flower bud had been
initiated, but that exposure of vegetative stages prior to floral initiation did not
further enhance the effect of irradiation on the number of seed per fruit.

From these results it can be said that the reduction of viable seed set by
chronic radiation through entire life span is mainly induced at three stages, i. e.,
floral initiation, meiotic division and early stage of embryogenesis.

II Radiosensitivity of Gametic Stage

The purpose of this experiment was to estimate the radiosensitivities judged
by gametic abortion following the exposure through gametogenesis including
meiotic division of spore mother cells, which was shown to be one of the three
radiosensitive stages in the abovementioned experiment using tomato plants, and
their relationship with the interphase chromosome volume. Experiments for
pollen abortion were performed at first using five species, and then the relation-
ship between pollen abortion and ovule abortion and their influence to seed set
were determined using tomato plants.

A. Pollen Abortion

The effects of chronic gamma irradiation on the percentage of aborted pollen
were observed in _Arabidopsis thaliana, Antirrhinum majus, Lycopersicum escul-
tenum, Nigella damascena_ and _Tradescantia paludosa_, representing a wide range
of radiosensitivity as predicted by their interphase chromosome volumes (ICVs).
Pollen abortion was judged by the number of collapsed cells and/or by degree of
staining. Pollen abortion was increased most drastically in the period from the
differentiation of pollen mother cells to the end of meiosis, termed the "pollen-
sensitive period" The regression of daily exposures producing 50 per cent pollen abortion after the exposure through "pollen-sensitive period" against ICVs of shoot meristematic cells showed a distinct correlation between sensitivity and chromosome size when both values were plotted in logarithmic scale (Fig. 5), but the slope (-1.426) indicated that the relationship is not purely proportional but that cells with large chromosomes were more sensitive than which had been expected from the -1 slope, and those with small chromosomes were more resis-
Fig. 6. Regression of accumulated exposure administered within the period from pollen mother cell differentiation to the end of meiosis (i.e. pollen-sensitive period) which increased pollen abortion to 50 per sent in five species plotted against interphase chromosome volumes (data from Table 2) (Yamakawa and Sparrow, 1966)
Table 2. Summary of somatic interphase chromosome volume, estimated number of days from differentiation of pollen mother cells to the end of meiotic division, and daily exposure required to increase pollen abortion to 50 per cent

<table>
<thead>
<tr>
<th>Plant number, species and somatic chromosome number (2n)</th>
<th>Average nuclear volume of shoot meristems (μm²± S. E.)</th>
<th>Average interphase chromosome volume (μm²± S. E.)</th>
<th>Estimated no. of days from differentiation of P.M.C. to the end of meiosis</th>
<th>Exposure for 50% pollen abortion</th>
<th>Accumulated exposure (kR) in the period stated in the 4th column</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. <em>Tradescantia paludosa</em> (B2-2)(12)</td>
<td>833.0±56.69</td>
<td>69.4±4.72</td>
<td>14.0</td>
<td>10.4</td>
<td>0.146</td>
</tr>
<tr>
<td>2. <em>Nigella damascena</em> HV Miss Jekyll(12)</td>
<td>480.3±16.18</td>
<td>40.0±1.35</td>
<td>8.0</td>
<td>26.3</td>
<td>0.210</td>
</tr>
<tr>
<td>3. <em>Lycopersicum esculentum</em> HV Shugyoku(24)</td>
<td>178.6±8.09</td>
<td>7.4±0.34</td>
<td>8.0</td>
<td>202.0</td>
<td>1.620</td>
</tr>
<tr>
<td>4. <em>Antirrhinum majus</em> (S-21)(16)</td>
<td>48.4±2.73</td>
<td>3.0±0.17</td>
<td>3.5</td>
<td>1190.0</td>
<td>4.170</td>
</tr>
<tr>
<td>5. <em>Arabidopsis thaliana</em> (10)</td>
<td>19.3±1.27</td>
<td>1.9±0.13</td>
<td>4.0</td>
<td>1650.0</td>
<td>6.600</td>
</tr>
</tbody>
</table>

(Yamakawa and Sparrow, 1966)

tant than expected. The duration of the pollen-sensitive period was longer for plants with large nuclei than for plants with small nuclei (Table 2), and when this was corrected and the total exposures accumulated within this period were plotted against ICVs, a clear regression curve with a slope of -1.098 was obtained (Fig. 6). This fitted slope is not significantly different from that of -1. A more detailed description of abovementioned experiment is given elsewhere (26).

B Correlation of Pollen Abortion Estimated by Cotton Blue Staining, with Pollen and Ovule Fertility Estimated by Productivity of Viable Seed

In the experiment (A) mentioned above, the effects of chronic gamma irradiation on microsporogenesis were judged by percentage of aborted pollen grains observed by staining the matured pollen with cotton blue. The problem of this simple test was that percentage of actually inviable pollen grains may be higher than that of aborted pollen grains determined in this way. Because there are many papers reporting that the irradiated grains lose their function to produce viable seeds even with an exposure which does not affect their appearance and germination rate. For example, with tomato plants, Nishiyama and Tsukuda
(1961) have reported that pollen grains of *Lycopersicum pimpinellifolium* irradiated with 10 kR of X or gamma rays, had their fertilizing function including development of seeds and fruits seriously disturbed, although they germinated well on agar-agar medium even with 100 kR. Brock and Franklin (1966) also reported that pollen grains of *Lycopersicum pimpinellifolium* reduced seed set by 80 per cent when irradiated with 5 kR of gamma rays. Those experiments were both acute exposure to matured pollen grains. Since, in the experiment presented here, radiations were administered chronically throughout microsporogenesis from the differentiation of pollen mother cells, so it is reasonable to expect that the effects of radiations at such early stages of microsporogenesis as differentiation and meiotic division of pollen mother cells, result in abortions of pollen grains judged by staining. The effects of radiation after meiotic division, however, may reduce the fertilizing functions of pollen grains even if they can be stained normally.

This experiment was undertaken in order to clarify the relationship between pollen abortion judged by staining and pollen fertility as evaluated by seed set. The effects of gamma irradiation on macrosporogenesis were also investigated by measuring ovule fertility, and were compared with those on microsporogenesis.

1. **Materials and Methods**

*Lycopersicum esculentum*, horticultural variety "Shugyoku", the same one as used in experiment (A), was used.

**Method to determine pollen abortion, pollen fertility and ovule fertility.** The seeds of plants were sown on March 20 in warm nursery bed, and two months later eighty plants showing uniform growth were selected for the experiment. Thirty plants including ten control plants were irradiated from April 22, when the first flower buds were 10—15 days before anthesis, to May 7. Since only those flowers which opened on May 10 and 11 were used, the flower buds had been exposed 19 or 18 days before anthesis through pollensensitive period described in the experiment (A). Two exposure rates, 100 and 200 R/day, were adopted which caused approximately 80 and 50 per cent pollen abortion respectively by chronic exposure throughout pollen-sensitive period in the experiment (A), and ten plants were used for each exposure rate. Other forty plants remained unirradiated, ten of them were used in order to provide unirradiated pollen with which irradiated flowers were pollinated, and thirty others were used to provide unirradiated ovules which were pollinated with irradiated pollen to check its fertility. In the irradiated plants, the tips of united anthers covering stigmas had been removed by forceps one or two days before anthesis, in order to prevent self pollination. Since a tomato flower has five or six anthers, they were
all removed with corolla on the day of anthesis and were divided into two. One of them was used for pollen abortion test and the pollen from another part of anthers was pollinated to unirradiated flower which had been castrated one or two days before anthesis.

Pollen abortion was judged in the same way as mentioned in the experiment (A), and pollen and ovule fertility were judged by the number of seeds obtained from fruits which were pollinated with irradiated pollen and with unirradiated pollen, respectively. Thus, pollen abortion, pollen fertility and ovule fertility can all be measured in each flower at the same time, making the comparison of these three end points easy and complete.

2. Results

Pollen abortion rates were 97.48±0.02, 81.27±1.51 and 58.83±1.67 per cent at control, 100 and 200 R/day respectively. These results roughly agree with the result of the experiment (A), although a little less abortion was observed in the present experiment.

Function of irradiated pollen. The data of fruit set, fruit weight and seed set of unirradiated flowers pollinated with irradiated pollen were summarized in Table 3. Fruit setting per cent and fruit weight remained high regardless of exposure rate and pollen abortion rate, and it is very interesting that the number of seeds per fruit is not influenced by them.

Ovule fertility. The effects of radiation on irradiated flowers were summarized

### Table 3. Fruit set, fruit weight and seed set of unirradiated flowers pollinated with irradiated pollen

<table>
<thead>
<tr>
<th>Daily exposure (R/day)</th>
<th>No. of flowers pollinated</th>
<th>Normal pollen of irradiated flowers (± S.E.)</th>
<th>Fruit setting (%)</th>
<th>Fruit weight (Gr. ± S.E.)</th>
<th>No. of seeds per fruit (± S. E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19</td>
<td>97.48±0.02</td>
<td>89.5</td>
<td>12.6±1.3</td>
<td>23.4±4.2</td>
</tr>
<tr>
<td>100</td>
<td>22</td>
<td>81.27±1.51</td>
<td>80.9</td>
<td>14.1±1.7</td>
<td>28.3±6.3</td>
</tr>
<tr>
<td>200</td>
<td>16</td>
<td>58.83±1.67</td>
<td>100.0</td>
<td>12.1±1.3</td>
<td>21.4±9.6</td>
</tr>
</tbody>
</table>

### Table 4. Fruit set, fruit weight and seed set of irradiated flowers pollinated with unirradiated pollen

<table>
<thead>
<tr>
<th>Daily exposure (R/day)</th>
<th>Fruit setting (%)</th>
<th>Fruit weight (Gr. ± S.E.)</th>
<th>No. of seeds per fruit (± S. E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>94.6</td>
<td>20.1±1.0</td>
<td>50.9±3.9</td>
</tr>
<tr>
<td>100</td>
<td>90.9</td>
<td>19.4±1.2</td>
<td>35.9±3.9</td>
</tr>
<tr>
<td>200</td>
<td>93.1</td>
<td>15.8±1.1</td>
<td>21.7±2.8</td>
</tr>
</tbody>
</table>
in Table 4. Although fruit setting is not affected, fruit weight was decreased by exposure, especially at 200 R/day. Since it is well known that the fruit size of tomato is not affected by the number of fertilized eggs and seed development as can be seen in parthenogenetic development by hormon treatment, it is likely that the reduction of fruit size is not caused by ovule sterility but by the radiation damage in floral organ development. In the present experiment, the flower buds were exposed from 19 or 18 days before anthesis in order to be exposed through entire pollen sensitive period, and at that time, pollen mother cells have not differentiated yet as had been described by Yamakawa and Sparrow (1966). This fact agrees with the above consideration that the reduction of fruit size is the effect of exposure in later stage of floral organ development, and this consideration also agrees with the fact that at 100 R/day, the fruit weight was just slightly reduced in spite of considerable reduction of seed set, because the floral organ development is sharply disturbed at exposure rates over 100 R/day as will be shown later (Fig. 13).

In order to separate the radiation effect on ovule fertility from that in floral organ developmental stage, the number of seed was plotted against fruit weight in Fig. 7. Clear regressions could be found in each of three exposure rates, and since the individual slopes of three regression lines did not differ significantly.
each other, they were drawn with the best slope of 1.17. From this figure, it can be seen that at 100 R/day the ovule fertility, estimated by number of seeds, decreases to 71.5 per cent of control, and at 200 R/day to 61.1 per cent. Since these values are constant regardless of fruit weight, the radiation effect on ovule fertility can be estimated independently of fruit weight, which is the result of exposure in floral developmental stage.

Pollen abortion at 100 and 200 R/day were 83.3 and 60.3 per cent of control, respectively. When these values are compared with ovule fertilities abovementioned, it can be said that the micro- and macro-sporogenesis have approximately the same level of radiosensitivity, and in both cases the abortion increases to 50 per cent at around 200 R/day.

3. Discussion

In this experiment, the fruit set and seed set of unirradiated flowers which were pollinated with pollen from irradiated plants were not affected regardless of pollen abortion rate. Nishiyama and Tsukuda (1961) have reported that X and gamma irradiation of matured pollen decreased the fruit-setting and seed-setting percentage of flowers being pollinated with irradiated pollen even with such small exposure as not to affect pollen germination rate. They suggested two possibilities: (1) Irradiated pollen grains fail to fertilize egg cells in usual manner and the development of fruits is due to parthenocarpy caused by stimuli of pollen-tube growth through the style or of pollen nuclei. (2) Fertilization takes place in normal manner but fertilized egg cells or endosperms soon fail to develope, owing to damage or loss of pollen function of nuclei. The author thinks the later possibility is more likely by two reasons: (1) numerous pollen grains can be pollinated on the stigma, and even if most of them lose their fertilizing function, there must be still enough number of functional pollen grains to fertilize ovules in a fruit because the ovules are quite few if compared with pollen grains. (2) In L. pimpinellifolium the ripe pollen has two nuclei, a vegetative and generative nucleus, and in most binucleate pollen species the chromosomes effectively double in the generative cell prior to shedding (Brewbaker and Emery, 1962). Since it has been known that cells which are irradiated while undergoing division do not display the full extent of chromosomal damage until they have passed through interphase and have divided again (Sparrow et al., 1952), it is likely that the mitosis of a generative nucleus can be performed normally, and the chromosomal damage become apparent after fertilization.

In the present experiment, the affected cells in early stages of microsporogenesis such as differentiation and meiotic division of pollen mother cells resulted in aborted pollen being identified by the degree of staining, and undoubtedly these
Fig. 8-9. Longitudinal sections comparing the size of pollen- and embryo-sac-mother cells in the same flower at 11-10 days before anthesis. The mother cells have reached late pachytene of meiotic prophase and pollen mother cells have an average nuclear volume of $601 \pm 15 \mu^3$. Both magnifications are $\times 740$. 
aborted pollen grains can not germinate. In other words, affected cells could be eliminated through the first and second meiotic divisions, microspore division and pollen germination, and the results of present experiment showed that the nonaborted and germinated pollen grains can fertilize the egg cells and allow the embryos to grow normally up to viable seeds.

From abovementioned consideration the reason of the difference between the results of two experiments can be explained in the following way. In the case of matured pollen irradiation, since elimination of affected cells does not take place by the time of fertilization, the effect of irradiation can be observed as the reduction of viable seeds per fruit. On the other hand, if the radiation is administered in early stages of microsporogenesis, affected cells can be eliminated through the following divisions and pollen germination, and once they germinate they have enough capacity to fertilize egg cells and allow the embryos to grow normally up to viable seeds, and if enough number of nonaborted pollen grains to fertilize the ovules in a flower were pollinated, no reduction of seed set could be observed.

Ovule sterility increased by approximately same manner as pollen abortion. In the experiment (A), differentiation of sexual organs of tomato was investigated histologically and it was found that meiotic divisions of pollen mother cells and embryosac mother cells progress simultaneously. The comparison of nuclear volumes of pollen and embryosac mother cells was also tried. Although the number of embryosac mother cells being observed clearly enough to measure their nuclear volumes were not sufficient to calculate the volumes statistically, they looked to have approximately same volumes as those of pollen mother cells as can be seen in Fig. 8 and 9, where pollen and embryosac mother cells in the same flower were shown respectively. These observation agrees with the results of present experiment that micro-and macro-sporogenesis have approximately the same level of radiosensitivity.

The result of the later experiment using Nigella, also suggested that micro- and macro-sporogenesis have the same level of radiosensitivity. In this species, the floral organ developmental stage and the zygotic stage are both quite radio-resistant if compared to the gametic stage as can been seen in Fig. 12. Since, no reduction was seen in the number of spore mother cells differentiated, which represents radiation effects during floral organ developmental stage, and zygotic fertility, up to the exposure rate of 30 R/day where gametic fertility and seed set decrease to approximately 40 per cent, it can be said that the seed set is affected only by the exposure during gametic stage. In Fig. 12, seed set and gametic fertility decrease in the same manner up to 30 R/day, although the
values of gametic fertility have kept slightly below those of seed set. This phenomenon completely agrees with the conclusions drawn in the present experiment, that is: (1) the seed set is not affected by the abortion rate of the pollen in the case the abortion rate is not so high as above 50 per cent. (2) the micro- and macro-sporogenesis have approximately same level of radiosensitivity.

III Correlation of Zygotic Abortion Induced by Chronic Gamma Irradiation and Interphase Chromosome Volume

In the present experiment, the radiosensitivity in zygotic stages, early stage of which including fertilization was found to be also one of the three radiosensitive stages, was investigated.

1. Materials and Methods

Five species, same ones as used in the experiment II- (A) with one exception, were selected as representing plants with a suitable range of interphase chromosome volume. Instead of Tradescantia paludosa used in the experiment II, which can seldom set seeds. Pisum sativum IV. Alasca was used in the present experiment.

Irradiation was carried out in the gamma greenhouse of Brookhaven National Laboratory in 1965.

Duration of exposure. The results of the experiment-I showed that early stage of embryogenesis has high radiosensitivity. From Fig. 3, the most radiosensitive period in tomato embryogenesis seemed to be around 10 days after anthesis, and according to Smith's histological study (Smith, 1935), the period corresponds to early proembryo stage. Mericle and Mericle (1957) have investigated the effects of X irradiation on embryogeny of barley. In their histological study, although the earliest proembryo stages showed less radiosensitivity than many of the later ones, there was, from few cell stages through differentiation, an inverse correlation between the frequency of effects and stage of embryo development at the time of irradiation. Furthermore, it was seen that while the earliest proembryo stages, such as one or two cell stages, are not as radiosensitive from a histological viewpoint as the later stages, they are the most radiosensitive with regard to germination-seedling effects.

From their results, it is likely that in embryogenesis there are not critical stages such as meiotic division in sporogenesis, but high radiosensitive period begins at fertilization and lasts for some duration through proembryo stages, and the embryos gradually lose their sensitivities as they differentiate and mature. Therefore, irradiation must be administered covering full periods from fertilization to seeds maturation, in order to estimate the effects of chronic exposure on embry-
genesis.

In the present experiments plants were grown in standard green house, and brought into gamma green house for exposure when they began to flower, and had been exposed until seed maturation. In *Arabidopsis* and *Antirrhinum*, only those flowers, which opened on the day of start of exposure, were used, and in other three species, the flowers which opened on the next day were also used. In the later cases, pollen and ovules were also irradiated for less than one day, but, since, as mentioned in the experiment II-(A), matured pollen and ovule are quite resistant to radiation, the effect of radiation before pollination could be neglected. Flowers were pollinated with unirradiated pollen except in the case of *Pisum sativum*, where the flowers were open pollinated because of difficulty of pollination.

In this experiment, the effect of chronic exposure through entire reproductive

![Graphs showing number of viable seeds obtained from 5 species](image)

Fig. 10. Number of viable seeds obtained from 5 species which had been chronically irradiated at various exposure rates throughout the reproductive stages from flower bud initiation to seed maturity (open circle), and from anthesis to seed maturation (closed circle).
stages on seed set was also investigated, in order to compare the effects of exposure through entire reproductive stages with those limited to zygotic stages, at the same time and in the same conditions although in some species this had already been done by Yamakawa and Sparrow (1965). The plants used for this purpose, had been exposed from young seedling stage before flower bud initiation up to seed maturation.

Exposure rates. Suitable range of exposure rates was determined for each species referring to their interphase chromosome volumes and regression lines drawn in Fig. 2. Lithium fluoride dosimeters (Cameron et al., 1961) were placed on each inflorescence of the close-in plants to determine the accurate dose of exposure that each bud received daily.

2. Results and Discussion

In Fig. 10 exposure-rate response curves of viable seeds were drawn for both exposure after pollination and exposure through reproductive stages. The curves of the form \( Y = 100 + ax + bx^2 \) were fitted by the method of least squares to the data, where \( Y \) is daily exposure causing 50 per cent viable seed number and \( X \) is interphase chromosome volume. In the case, where exposure was limited to after pollination, the number of ovules to be fertilized is equal in control and irradiated flowers, therefore, the percentage of viable seeds can be considered as the percentage of fertility in zygotic stages including fertilization. In the following discussion, for the simplicity of expression, the percentage of viable seeds of flowers exposed after pollination and that of flowers exposed through reproductive stages are called as percentage of zygotic fertility and that of seed set respectively. From Fig. 10, the exposure rates causing 50 per cent reduction of zygotic fertility

<table>
<thead>
<tr>
<th>Plant number and species</th>
<th>Avg. interphase chromosome volume ( (\mu^2 \pm S. E.) )</th>
<th>Daily exposure reduced for 50% zygotic fertility</th>
<th>50% seed set</th>
<th>50% seed set</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Nigella damascena</td>
<td>40.0±1.35</td>
<td>54.8</td>
<td>26.0</td>
<td></td>
</tr>
<tr>
<td>HV. Miss Jekyll</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Pisum sativum</td>
<td>15.0±0.77</td>
<td>98.0</td>
<td>57.2</td>
<td></td>
</tr>
<tr>
<td>HV. Allaska</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Lycopersicum esculentum</td>
<td>7.4±0.34</td>
<td>150.0</td>
<td>93.3</td>
<td></td>
</tr>
<tr>
<td>HV. Shinyoku</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Antirrhinum majus</td>
<td>3.0±0.17</td>
<td>862.0</td>
<td>213.0</td>
<td></td>
</tr>
<tr>
<td>(S-21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Arabidopsis thaliana</td>
<td>1.9±0.13</td>
<td>1610.0</td>
<td>1110.0</td>
<td></td>
</tr>
</tbody>
</table>
and seed set were estimated, and the results were summarized in Table 5 with the interphase chromosome volumes.

Fig. 11. Regressions of daily exposures required for 50 per cent zygotic abortion and 50% reduction of viable seed set in five species plotted against interphase chromosome volumes. (data from Table 9).

From Fig. 11, where the daily exposures required to reduce zygotic fertility and seed set to 50 per cent were plotted against interphase chromosome volume, a distinct correlations were found, as in the case of seed set and pollen abortion in previous experiments. The slopes were $-1.145$ and $-1.108$ respectively, which did not deviate significantly from $-1$, and the regression line of seed set did not significantly deviate from that of previous seed set experiment in Fig. 1.

IV Correlation of Disturbance In Floral Development by Chronic Gamma Irradiation and Interphase Chromosome Volume

Generating process of seed can be divided into three stages: (1) Developmental
stage of floral organ, from flower bud initiation to differentiation of spore mother cells. The effects of radiation on seed set in this stage can be estimated by the number of spore mother cells differentiated per flower. (2) Sporo-and gameto-

![Graph](image)

Fig. 12

![Graph](image)

Fig. 13

Figs. 12-15. Estimated number of embryo-sac mother cells (M) differentiated at various exposure rates. The values were calculated from three other exposure-rate response curves of seed set (S), gametic abortion (G) and zygotic abortion (Z). Fig. 12-Nigella damascena, Fig. 13-Lycopersicum esculentum, Fig. 14-Antirrhinum majus, Fig. 15-Arabidopsis thaliana.
genesis, from differentiation of spore mother cells to maturation of gametes. The effects of radiation on seed set in this stage can be estimated by the degree of gametic fertility. (3) Zygotic stage, from fertilization to seed maturation. The effects of radiation on seed set in this stage can be estimated by the degree of zygotic fertility. In the experiment II-(B), it was revealed that even if considerable portion of pollen was aborted in the process of microsporogenesis, still enough number of pollen grains, having function to fertilize the egg cells, remain,
resulting in no reduction of seed set of fruit pollinated with them. So, the effects of radiation on seed set in the stages before fertilization can be limited to macrosporogenesis.

From the above discussion, the relationship between the radiation effects in the abovementioned three stages and on final seed set can be shown by a formula \( (S) = (M) (G) (Z) \), where \( S \) is number of viable seeds finally produced, \( M \) is number of embryosac mother cells differentiated, \( G \) is female gametic fertility, i.e., ovule fertility, and \( Z \) is zygotic fertility. Since, in the experiment II (B), it was shown that micro- and macrosporogenesis have approximately the same level of radiosensitivity, in the following discussion, ovule fertility was substituted by pollen fertility, because scoring of pollen fertility is much easier than that of ovule fertility. Since, exposure rate response curves of all of \( S, G \) and \( Z \) in abovementioned formula were already drawn in four species, \( M \) could be calculated by dividing \( S \) by the product of \( G \) and \( Z \).

In figures 12 to 15, these calculated \( M \) values were plotted against daily

![Graph](image)

**Fig. 16.** Regression of daily exposure required for 50 per cent reduction of spore-mother-cell differentiation in five species plotted against interphase chromosome volumes (data from Table 6).
exposure. Other three curves already drawn for S, G and Z were presented again for comparison. From these curves the daily exposures causing 50 per cent reduction of embryosac mother cells were estimated. They were 38.5, 102.5, 236.0 and 1470.0 R/day in Nigella, Lycopersicum, Antirrhinum and Arabidopsis respectively, and these values were plotted against interphase chromosome volume in Fig. 16. A clear relationship was found again. The slope of the regression line is $-1.044$, which does not significantly deviate from a $-1$ slope.

Since, the seed set is influenced in the periods covering the floral developmental stages from flower bud initiation to spore mother cell’s differentiation, as described in the section I, the number of differentiated embryosac mother cells can be a measure of radiation effect, judged by seed set, in floral developmental stages.

V Relative Radiosensitivities of Three Developmental Stages, i.e.,
Floral Development, Gametogenesis and Embryogenesis under
Chronic Gamma Irradiation

In four species, the effect of radiation was investigated in all of three developmental stages and in entire reproductive stage. The results were summarized in Table 6. When the daily exposure required for 50 per cent effect in each of four end points was plotted against interphase chromosome volume, four regression lines could be drawn as beforementioned, and since the individual slope of four regression lines did not differ significantly from each other, they were drawn with the best common slope of $-1.17$ in Fig. 17. It can be seen from this figure that approximately same exposure rate causes 50 per cent gametic and

<table>
<thead>
<tr>
<th>Plant number and species</th>
<th>Avg. interphase chromosome volume ($\mu^3 \pm$ S. E.)</th>
<th>Spore mother cell differentiation* (R)</th>
<th>Gametic fertility** (R)</th>
<th>Zygotot fertility (R)</th>
<th>Viable seed set (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Nigella damascena</td>
<td>40.0 $\pm$ 1.35</td>
<td>34.9</td>
<td>12.3</td>
<td>26.3</td>
<td>41.6</td>
</tr>
<tr>
<td>3. Lycopersicum esculentum</td>
<td>7.4 $\pm$ 0.34</td>
<td>93.8</td>
<td>102.5</td>
<td>90.0</td>
<td>202.0</td>
</tr>
<tr>
<td>4. Antirrhinum majus</td>
<td>3.0 $\pm$ 0.17</td>
<td>165.0</td>
<td>236.0</td>
<td>505.0</td>
<td>1190.0</td>
</tr>
<tr>
<td>5. Arabidopsis thaliana</td>
<td>1.9 $\pm$ 0.13</td>
<td>1290.0</td>
<td>1470.0</td>
<td>720.0</td>
<td>1650.0</td>
</tr>
</tbody>
</table>

* Estimated from three exposure-rate response curves of gametic abortion, zygotic abortion and seed set.

** Estimated by pollen abortion.
zygotic abortion, and about 0.6 and 0.5 of it causes 50 per cent reduction of spore mother cells and viable seed set respectively. Since the seed set after the exposure through entire reproductive stages is affected by exposure in any of three developmental stages, it is natural that the lowest exposure rate is required for 50 per cent reduction of seed set. When other three regression lines are compared, it can be seen that floral organ developmental stage is more sensitive than gametic and zygotic stage to the radiation effect which was estimated by the reduction of spore mother cell differentiation. The relative radiosensitivity of these stages, however, varied with species. In *Antirrhinum* and *Lycopersicum*, floral developmental stage is more sensitive than gametic and zygotic stages as can be seen in Fig. 13 and 14. Especially in *Antirrhinum*, the floral developmental stage is so
sensitive that the seed set is mostly reduced in this stages. In *Arabidopsis*, approximately same exposure rates cause 50 per cent effects in three stages (Fig. 15). In *Nigella*, however, gametic stage is so sensitive that seed set reduction occurs mostly in this stage (Fig. 12). These results agree with the observation in the experiment II-(A), that in the case of three species (*Tradescantia, Lycopersicum* and *Antirrhinum*) the organ-forming stages of the young flowers were so sensitive that, if the exposure rate for increase of pollen abortion to 50 per cent was extended through this period, the flower would be lost or the anthers would be highly altered, however, in the case of two species (*Nigella* and *Arabidopsis*), the organ-forming stages were comparatively insensitive to the exposure rates required for 50 per cent pollen abortion.

The comparison of radiosensitivity of three stages were so far done using daily exposures causing 50 per cent effects, however, the pattern of exposure-rate response curve is quite different in three stages. From Fig. 12–15, it can be seen that the curves for gametic fertility is sigmoid having no threshold exposure rate, the curves for zygotic and floral developmental stages, however, have shoulder parts and sharply fall after threshold exposure rates. This tendency is more striking in floral developmental stage. From Fig. 18 where the data were replotted in order to illustrate the relative responsibility of the radiation effects in three

![Graphs A, B, C, D](image_url)

**Fig. 18.** The percentage of reduction of spore-mother-cell differentiation, gametic fertility and zygotic fertility at various rates of seed-set reduction.
stages for the reduction of seed set, it can generally be said that, in the case where the seed set is reduced slightly, the gametic sterility is mostly responsible for it, and as the seed set reduces further, the damages in zygotic and floral developmental stages have higher responsibility, and finally seed set is completely inhibited by the severe disturbance of floral organ development.

From above consideration, the relative sensitivity of three stages can not be determined only by the exposure rate causing 50 per cent effect. Thus, the daily exposure causing 20 per cent effect in each of four end points were plotted against interphase chromosome volume in the same way as Fig. 17, with the best common slope of -1.16. From Fig. 19, it can be seen that gametic stage is the most sensitive stage as expected from abovementioned comparison of three exposure-rate response curves. Zygotic stage was the most resistant again.

It may be concluded that seed set reduction begins by gametic abortion, and becomes severe by zygotic abortion and floral disturbance, and finally becomes zero by severe disturbance of floral organ development.

Fig. 19. Regressions comparing the daily exposure required for 20 per cent reduction of viable seed set, spore-mother-cell differentiation, gametic fertility and zygotic fertility (data from Table 6).
The reason why the patterns of exposure-rate response curves are different among three stages is unknown, but it might be explained as follows. Gametic abortion is caused mostly in three cell divisions, i.e., division of archesporial cells, the first and second meiotic divisions, as mentioned in the experiment II-(A). Since any damage in these three divisions results in gametic abortion without compensation effect, no threshold could be seen in the exposure-rate response curve of gametic abortion. Zygotic abortion and floral disturbance, however, are caused through many cell divisions and if a few cells are killed by radiation, other cells can divide to compensate them. This may be the reason why there are threshold exposure rates in their exposure-rate response curves.

The relative sensitivity of three developmental stages varied with species. In *Antirrhinum majus* floral developmental stage was very sensitive, and in *Nigella damascena* gametic stage was very sensitive. The reason causing this difference is unknown, and must be studied further.

**VI SUMMARY**

1. In tomato plants, three developmental stages were found to be highly radiosensitive. The first is floral organ developmental stage from flower bud initiation to differentiation of spore mother cells. The second is spore and gametogenesis from differentiation of spore mother cells to gamete maturation, including meiotic divisions. The third is zygotic stage from fertilization to seed maturation, including proembryo stage.

2. The effects of chronic gamma irradiation during three developmental stages on final seed set were observed in *Arabidopsis thaliana*, *Antirrhinum majus*, *Lycopersicum esculentum*, *Pisum sativum* *Nigella damascena* and *Tradescentia paludosa*, representing a wide range of radiosensitivity as predicted by their interphase chromosome volumes.

3. The regression of daily exposures producing 50 per cent pollen abortion against interphase chromosome volumes showed a distinct correlation between sensitivity and chromosome size.

4. In tomato plants, pollen and ovule abortions were induced by approximately the same exposure rate, and the seed set of unirradiated flowers which were pollinated with irradiated pollen were not affected regardless of pollen abortion rate.

5. A distinct correlation was also found between daily exposures required for 50 per cent zygotic abortion and interphase chromosome volumes.

6. Daily exposures required for 50 per cent reduction of spore mother cells were estimated for four species from exposure-rate response curves of seed set,
gametic fertility and zygotic fertility. They also showed fairly good regression against interphase chromosome volumes. They can be used as a criterion of radiosensitivity in floral organ developmental stage.

7. Sensitivities of three developmental stages were compared by their regression lines against interphase chromosome volumes. When daily exposures required for 50 per cent effects were compared, floral organ developmental stage was the most sensitive stage, but when those for 20 per cent effects were compared, gametic stage was the most sensitive stage, and in both cases, zygotic stage was the most resistant stage. This difference was expected from the difference of the patterns of exposure-rate response curves of three end points. The gametic abortion was induced even at slight exposure rate, floral organ development and zygotic fertility, on the contrary, remained high at lower exposure rate, but decreased sharply when the exposure rate exceeds the threshold values.

Acknowledgment

The author wishes to express his sincere gratitude to Dr. A. H. Sparrow of Brookhaven National Laboratory for providing much valuable data including the list of interphase chromosome volumes of a number of species, and to Director K. Kawara of the Institute of Radiation Breeding for a number of valuable suggestions. The author also indebted to Dr. S. Tanaka, Mr. A. Yamashita, Mr. Y. Takagi and Mr. I. Yamaguchi for their permission to present their seed-set data for Oryza, Hordeum, Phaseolus, Pisum and Lycopersicum. Finally, the author is very grateful to Prof. N. Sugiyama, Prof. T. Matsuo and Dr. H. Yamaguchi of University of Tokyo for the valuable suggestions and criticisms in preparing manuscript.

References

クローニック照射における花器，配偶子および胚形成期の相対的放射線感受性について

山川邦夫
（農林省放射線育種場）

クローニック照射において，栄養生長をかすかに抑制する線量率と種子生産力を半減させす線量率を比較すると，後者は前者の約1/2であり，生殖生長期間が栄養生長期間に比べはるかに放射線感受性が高いことについては既に報告した（Yamakawa and Sparrow, 1965）。またトマトを用いた予備実験の結果，クローニック照射による種子生産力の低下は主として花芽分化以後の照射によるものであることがわたった。

花芽分化から種子稔実にいたる期間を“全生殖生長期”とよれば，これを次の三時期にわけることができる。1)花芽分化から花器形成をへて生殖原細胞の分化に至る期間。この時期での照射の種子生産におよぼす影響は，やくや難しいの発達を阻害し，その中に分化する生殖原細胞の数を減少させるる。2)生殖原細胞の分化から卵数分裂をへて配偶子ができるまでの期間。この時期の照射の種子生産におよぼす影響は，花粉粒や胚珠の不稔としてあらわれる。3)受精から前胚期をへて種子成熟にいたる期間。この時期の照射の種子生産におよぼす影響は，受精卵あたりの成熟種子数の低下という形であろう。ここでは1)を花器形成期，2)を配偶子形成期，3)を胚形成期とよぶ。本実験はガンマ線のクローニック照射による種子生産力低下の機構を明らかにする目的で行なわれた。各時期の感受性の比較はそれぞれの効果をひきおこすに必要な線量率の染色体容積に対する回帰直線の比較により行った。

A) 配偶子形成期照射による配偶子不稔について

花粉異常 前報（Yamakawa and Sparrow, 1966）において詳述したように，50％花粉異常を生じめる線量率における花粉母細胞の分化から減数分裂終了までの期間に蓄積された線量率は，それぞれの種の染色体容積に対し明瞭な回帰を示し，その係数は -1 より有意にはさられなかった。

照射花粉の受精能力および胚珠稔性；上記の試験での異常花粉とは，染色観察による形態的異常粒であった。その際一見正常とみられた花粉の受精力について，トマトで試験したところ，異常花粉の率いかんにかかわらず，これを受粉した花の着果率や種子数には変動がなく，このことから正常にみえる花粉粒は，卵を受精し，胚を成熟種子まで発達させる能力を有しているものと推定された。同じくトマトを用いて交配実験を行なった結果，胚珠の感受性も花粉のそれとはほぼ同様であることがわかった。

B) 胚形成期照射による胚の不稔について

開花直後から種子成熟までのクローニック照射により種子数を半減させる線量率と染色体容積との関係を調べ，両者の間に明瞭な相関を認めた。また既報の実験（Yamakawa and Sparrow, 1965）と重複するが，この実験に供試した5種について全生殖生長期照射
射による種子半減線量も同時に検定された。

C）花器形成期照射による母細胞分化数の減少について
全生殖生長期間照射による種子数S，母細胞の分化数をM，配子不稔をG，胚不稔をZとすれば，

\[ S = M \times G \times Z \]

となる。（Aの実験結果から配子不稔は胚珠不稔のみを考えればよく，これは花粉不稔によって代用される。A），B）両実験の結果，4種については，S，G，Zともにわかっているので，Mは計算できる。このように推定された母細胞分化数を半減せしめる線量率を，それぞれの染色体容積と比較したところ，やはり両者の間に明瞭な相関がみられた。

D）三時期の相対的放射線感受性について
以上，A)，B)，C）の三時期および全生殖生長期間照射について得た50％効果の4本
の回帰直線を比較すると，花器形成期がもっとも感受性が高く，配子および胚形成期は
ほぼ同様の感受性を有していた。しかし，三時期における照射線量率の効果曲線を比較する
と，配子不稔がもっとも低線量率でもおこり，他の二つは一定以上の線量率になって
はじめて急激に減少した。そこで今までの50％の効果の線量率にかかわって20％の結果の線
量率について，同様に回帰直線で比較したところ，今度は配子形成期がもっとも感受性
が高く，花器形成期，胚形成期の順であった。これらの結果から，種子産生力の低下は比
較的低線量率では配子不稔による場合が多く，線量率が高くなるにつれて他の二時期，
とくに花器形成期の障害による率が高くなることがわかった。また，三時期の感受性は種
によって非常にことなり，きんぎおそうは花器形成期が，くろたねそうは配子形成期が
とくに感受性が高かった。

質 疑 応 答

生井 放射線感受性の差を核容積の差で見る場合に，種を多く扱うよりも，同種内でも

tunica 層細胞核の大きさは品種間で違っていることもありますから，まず同一種内の品
種についてお考えすべきではないでしょうか。

山川 私は放射線感受性を核容積と関係づけるには，十倍とか百倍のように広いレンジの
ものを使わせたいと考えております。品種間差異のような場合には，放射線感受性を支配
する他の要素を考えるべきだと思う。

藤井 Brewbaker 氏が様々な植物の花粉照射について報告したところによると，致
死率20％程度は花粉の生存曲線の“肩”のあたりにあります。したがって，直線領域に入
ってからの生存曲線で見る方が，より正確ではないかと思います。

山川氏は花粉の不稔などを調べる際に20％を基準にしていますが，放射線感受性の差を
比較する場合には，50％程度の激しい障害を基準として使うべきではないでしょうか。

山川 種子数などは不稔率50％を基準にした際にうまくいくと思われますが，花粉の場
合には，50％あたりが調査可能な限界であって，それを越すと，花自体が激しい障害を受
けるため，花粉稔性を調査することは技術的にも困難です。
昭和42年10月25日 印 刷
昭和42年10月30日 発 行

農 林 省 放 射 線 育 種 場
茨 城 県 那 琵 郡 大 宮 町

印刷所 三報社印刷株式会社
印刷者 永井 佐 波 太 郎
東京都江東区亀戸町 5 の 7