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Japan
MUTANTS IN PHYSIOLOGICAL RESEARCH OF CROP PLANTS

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Institute of Radiation Breeding
NIAS MAF
Ohmiya-machi, Ibaraki-ken
Japan
Participants at the Symposium

Abe, S.  
Miyagi Agricultural Experiment Station, Furukawa Branch

Aiga, I.  
The National Institute of Environmental Studies

Akabanee, M.  
Tochigi Agricultural Experiment Station, Kanuma Branch

Arima, K.  
Aomori Prefectural Experiment Station of Agriculture

Danbara, H.  
National Institute of Animal Industry

Endo, O.  
Bio-environment Laboratory, Central Research Institute of Electric Power

Fujii, T.  
National Institute of Genetics

Fujimoto, M.  
Faculty of Agriculture, Kyoto University

Fujita, H.  
Institute of Radiation Breeding, N.I.A.S.

Fukui, K.  
Faculty of Agriculture, Kyoto University

Furukoshi, T.  
Kanto Forest Tree Breeding Station

Gonai, H.  
T. Sakata and Company

Hagiya, K.  
Faculty of Agriculture, Niigata University

Handa, T.  
Kanto Forest Tree Breeding Station

Hasegawa, H.  
Radiation Center of Osaka Prefecture

Hata, T.  
Nagano Agricultural Experiment Station

Hirabayashi, H.  
Keisei Rose Research Institute

Hiraiwa, S.  
Institute of Radiation Breeding, N.I.A.S.

Hirano, T.  
Akita Prefectural College of Agriculture

Hori, K.  
Kanto Forest Tree Breeding Station

Ikeda, F.  
Institute of Radiation Breeding, N.I.A.S.

Inagaki, M.  
Central Agricultural Experiment Station

Ishida, Y.  
Peanut Breeding Laboratory, Chiba-ken Agricultural Experiment Station

Ishihara, M.  
Ibaraki Agricultural Experiment Station

Ishii, R.  
Peanut Breeding Laboratory, Chiba-ken Agricultural Experiment Station

Ito, J.  
Tottori Agricultural Experiment Station

Iwaki, H.  
National Institute of Agricultural Sciences

Kariya, K.  
Institute of Radiation Breeding, N.I.A.S.

Kataoka, K.  
Faculty of Agriculture, Tamagawa University

Katayama, T.  
Faculty of Agriculture, Kyushu University

Katsuta, M.  
The Government Forest Experiment Station

Kawai, T.  
National Grassland Research Institute

Kinoshita, T.  
Faculty of Agriculture, Hokkaido University
KOBAYASHI, T. Faculty of Literature and Science, Toyama University
KOWYAMA, Y. Faculty of Agriculture, Meiji University
KUDO, K. The Research Institute of Fermentation, Yamanashi University
KUKIMURA, H. Institute of Radiation Breeding, N.I.A.S.
KURAISHI, S. Department of Biology, College of General Education, University of Tokyo
KUWABARA, T. Central Agricultural Experiment Station
KUWADA, H. Faculty of Agriculture, Kagawa University
MAETA, T. Institute of Radiation Breeding, N.I.A.S.
MATSUNAKA, S. National Institute of Agricultural Sciences
MATSUO, T. Faculty of Agriculture, Tamagawa University
MATSUOKA, K. Tokyo University of Agriculture
MIYAMOTO, S. National Institute of Animal Industry
MIYAZAKI, S. T. Sakata and Company
MORI, S. Faculty of Agriculture, Kyoto Prefectural University
NAGASAKA, H. Kanto Forest Tree Breeding Station
NAGATA, N. Institute of Radiation Breeding, N.I.A.S.
NAKAJIMA, K. Sericultural Experiment Station
NAKAYAMA, A. National Research Institute of Tea
NAKAYAMA, S. Ibaraki-ken Experimental Station of Animal Industry
NITSUMA, Y. Ibaraki Agricultural Experiment Station
NISHIDA, T. Fruit Tree Research Station
OKUNO, K. National Institute of Agricultural Sciences
OKUTSU, Y. Ibaraki Agricultural Experiment Station
ONO, S. Ibaraki Agricultural Experiment Station
ONOZAWA, Y. Faculty of Agriculture, Ibaraki University
OSONE, K. Faculty of Agriculture, University of Tokyo
OZAWA, H. Radioisotope Research Institute, Tokyo University of Agriculture
SARA, H. National Institute of Agricultural Sciences
SAMATA, Y. Faculty of Agriculture, Tamagawa University
SATO, H. Faculty of Agriculture, Kyushu University
SASAKI, M. Faculty of Agriculture, Tottori University
SHIMIZU, Y. Faculty of Agriculture, Kyoto University
TABUCHI, K. Kanto Forest Tree Breeding Station
TAKARA, T. Kaneko Seeds Co., Ltd.
TAKEDA, K. Faculty of Agriculture, Hirosaki University
TAKAGI, Y. Faculty of Agriculture, Saga University
Takahashi, M. Miyagi Prefectural Agricultural Research Center
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takahashi, N.</td>
<td>Iwate Prefectural Agricultural Experiment Station</td>
</tr>
<tr>
<td>Takato, S.</td>
<td>Institute of Radiation Breeding, N.I.A.S.</td>
</tr>
<tr>
<td>Takayanagi, S.</td>
<td>National Institute of Agricultural Sciences</td>
</tr>
<tr>
<td>Tanaka, S.</td>
<td>Institute of Radiation Breeding, N.I.A.S.</td>
</tr>
<tr>
<td>Tojyo, I.</td>
<td>Sericultural Experiment Station, Tohoku Branch</td>
</tr>
<tr>
<td>Tsunoda, S.</td>
<td>Faculty of Agriculture, Tohoku University</td>
</tr>
<tr>
<td>Tsuyuki, Y.</td>
<td>Faculty of Agriculture, Tamagawa University</td>
</tr>
<tr>
<td>Ukai, Y.</td>
<td>Institute of Radiation Breeding, N.I.A.S.</td>
</tr>
<tr>
<td>Umeki, S.</td>
<td>Faculty of Agriculture, Tamagawa University</td>
</tr>
<tr>
<td>Watanabe, M.</td>
<td>YAMAGATA Prefectural Agricultural Experiment Station, Shonai Branch</td>
</tr>
<tr>
<td>Watanabe, Y.</td>
<td>Sericultural Experiment Station, Tohoku Branch</td>
</tr>
<tr>
<td>Watanabe, Y.</td>
<td>National Institute of Agricultural Sciences</td>
</tr>
<tr>
<td>Yaguchi, N.</td>
<td>Keisei Rose Research Institute</td>
</tr>
<tr>
<td>Yamaguchi, H.</td>
<td>Faculty of Agriculture, University of Tokyo</td>
</tr>
<tr>
<td>Yamaguchi, I.</td>
<td>Institute of Radiation Breeding, N.I.A.S.</td>
</tr>
<tr>
<td>Yamashita, A.</td>
<td>Institute of Radiation Breeding, N.I.A.S.</td>
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<td>Yonezawa, K.</td>
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<tr>
<td>Yoshida, H.</td>
<td>Central Agricultural Experiment Station</td>
</tr>
<tr>
<td>Yoshida, T.</td>
<td>Fruit Tree Research Station, Okitsu Branch</td>
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</table>
FORWORD

After the discovery of artificial induction of mutations, a large number of induced mutants have been obtained. In plant science a few of mutant have contributed to crop improvement and some others have been used as genetic markers. Most of remained mutants have been buried in oblivion without releasing to scientists of other field as available stocks.

This is very unfortunate in developing the plant science, when considering that a surprising advances of biochemistry and molecular biology in microorganism has partly been established by using mutant strains. In our country, the utilization of mutant in the basic research in plant science is rare, because the systematic collection of induced mutant stocks has not yet been established and the organized or personnel communication among plant scientists of different field, is limited.

The Symposium Committee hope that the invited lectures will impress the importance of mutants in plant science and stimulate the active cooperation of genetisists with physiologists. The Committee wishes to thank all those who contributed to the Symposium, the speakers, the chairmen and the staff members who organized this meeting.

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........................................................................................................S. TSUNODA and P. R. KUMAR

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Hormonal aspect of dwarfism.................................................................S. KURASHI

Utilization of artificial mutants for fundamental researches on herbicides
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Temperature sensitive characteristics in the phenotypic manifestation of rice chloroplast mutants.............................I. AIGA, T. OMURA and H. SATO

Mutants of rice, soybean and vegetables........S. TANAKA, N. NAGATA and S. HIRAIWA

Brief descriptions on mutants in vegetatively propagated and tree crops
...........................................................................................................H. UKUMURA, F. IKEDA, H. FUJITA and T. MAETA

Discussion Chairman M. SASAKI and K. OSONE
STUDIES ON A PARTIALLY ASYNAPTIC MUTANT
AND UPRIGHT, LEAF-AREAL
MUTANTS OF RICE

Hikoyuki Yamaguchi
Laboratory of Radiation Genetics, Faculty of Agriculture,
The University of Tokyo, Tokyo 113

Masayuki Watanabe, Shin-ichi Sato
and Yoshinori Kanbayashi
Shonai Branch Station, Yamagata Prefecture Agricultural
Experiment Station, Fujishima, Yamagata 999-76

Mutants have contributed greatly to understanding gene action and unraveling complex metabolic pathways. Moreover, the introduction of mutants into the storehouse of genetic materials leads to investigation of new problems.

In this report, firstly, occurring some repair system after gamma irradiation would be revealed by using a partially asynaptic mutant of rice, and secondly the upright, leaf-areal mutants induced in rice by gamma radiation were agronomically evaluated.

A partially asynaptic mutant

In the previous paper of this symposium (Yamaguchi, 1972), I indicated that the damage produced by the absorption of the radiation or the diffusion of the chemical mutagens in barley and rice is potentially capable of leading to mutation, and that there are some sort of reversibility in the mutation process. Due to the evidence that almost no mutation could be induced by irradiating with gamma rays or treating with EMS in post-duplication interphase, $G_2$, it was assumed that the time of the terminal event for mutagenesis is mainly at DNA replication interphase, $S$, and that almost all the initial lesions are potentially reparable.

The repair mechanisms of altered DNA are fairly well understood for microorganisms and mammalian cell lines than those in plants. The two main repair pathways have been described for *Escherichia coli*; namely, the excision-resynthesis and post-replication (recombination) repair pathways. This is based on the fact that recombination-deficient (*rec*−) mutation as well as the *nvr*− mutation of *E. coli*
gave a further increase in radiation sensitivity (Howard-Flanders and Boyce, 1966).

In *Drosophila melanogaster*, c3G+ allele is responsible only for the formation of the synaptonemal complex in the oocytes, so that the c3G− allele did not produce it. When stage-7 and stage-14 oocytes and mature sperm of such c3G mutant were X-rayed, it was demonstrated that it was more radiosensitive than the wild type (Watson, 1969; 1972). This indicates that there is a partial overlap between the function of repair and recombination.

Somatic association is the phenomenon that homologous chromosomes in somatic cells lie closer together than would be expected by random arrangement. It has been observed in the root tip cells of wheat (Feldman et al., 1966), oats (Sadasivaiah et al., 1969; Dubuc and McGinnis, 1970; Thomas, 1973), barley (Fedak and Helgason, 1970; Yoshida and Yamaguchi, 1973), and rye (Yoshida et al., 1974). In particular, Thomas (1973) found that the loose association of homologous chromosomes in somatic cell was correlated with the failure of chromosome pairing at meiosis in asynaptic genotypes of *Avena sativa*. It is expected, therefore, that in asynaptic strain its homologous chromosomes fail to lie closely in the nucleus of somatic cell so that recombination is impossible to take place.

The experiments were designed to clarify whether the partially asynaptic mutant is more radiosensitive than wild type: it affect the repair process occurring after irradiation (Yamaguchi, 1974). Seeds of a partially asynaptic mutant, K-648, which appeared spontaneously, were kindly supplied by Prof. T. Katayama of Kyushu University. Two to eight univalents have been observed at the first metaphase of meiosis in this partially asynaptic mutant (Katayama, 1966). Because having been unknown its parent variety, a cultivar Kinmaze was used as wild type.

Dry seeds (about 12% moisture content) of K-648 and Kinmaze were exposed to 0, 5, 10, 20 and 30 kR of 137Cs gamma rays. The number of irradiated seeds was 300 and 600 for Kinmaze and K-648, respectively. Immediately after irradiation, the seeds were soaked in air-saturated water. Forty days-old seedlings were transplanted to the field, where the survival rate was determined at maturity. Field survival was significantly reduced in a partially asynaptic mutant compared with a wild type. Four panicles were harvested from early emerged tillers including main stem on each M1 plant, and the seed sterilities were determined on 100 panicles of each treatment. The mean seed setting was slightly less in K-648 than in Kinmaze. Frequencies of chlorophyll mutations were scored as the number of mutants per 1,000 M2 seedlings, and plotted against radiation exposure (Fig. 1). The frequency of mutations in K-648 increased exponentially with the exposure, whereas the yield of mutations in Kinmaze did linearly. More mutations, therefore, were induced in a partially asynaptic mutant than in a wild type with higher exposures.
From these results, it is evident that a partially asynaptic mutant was more radiosensitive than the wild type. This fact could be taken as indicating that there is a common basis for repair and recombination mechanisms even in higher plant. Recently, using an X-ray sensitive mutant, *rad 51*, which was defective in genetic recombination, Hama-Inaba and Saeki (1975) have obtained the result suggesting that the budding radioresistance of yeast cells is responsible for the chromosomal recombination.

Based on a differential incidence of chlorophyll mutations between a partially asynaptic mutant and a normal wild type, the following conclusion is presented: the repair with chromosomal recombination has much more fidelity and seemingly does not contribute to the induction of mutants.
Upright, leaf-areal mutants

The maximum rate of dry-matter production in plant at a given climate conditions is obtained with an optimum leaf-areal index (LAI) of the populations. If plants are grown vigorously under high temperature with heavy application of fertilizers, the leaf-areal index exceeds the optimum: their leaves shade with one another and the resultant grain yield reduces. Consequently, it has been emphasized that varieties with short and erect leaves exhibit less mutual shading and are adaptable to high levels of fertilizer application (Jennings, 1964; Donald, 1968). Based on such idea, these plant-type mutants were searched after chronic gamma irradiation, and the experiments were performed to evaluate them agronomically.

1) Breeding of mutant strains having short and erect leaves

Eight seeds of a cultivar Hatsunishiki of paddy rice were planted in a gamma field of the Institute of Radiation Breeding, Ibaraki, Japan. The resultant plants were exposed from seed-time to maturity with 200 R per day. In the next generation (M₃), 1,474 plants were allowed to grow, and eight albino mutants were observed at the seedling stage. A dwarf mutant having extremely narrow and erect leaves, referred as NF-1, was found among the M₃ progenies from 33 plants which were preliminarily selected in the previous generation. Because of short panicles of this NF-1 mutant, a lower grain yield was expected.

Improvement for productivity of this upright, leaf-areal mutant was initiated with the cross of NF-1 and Meitoku 5. The latter is vigorous in the vegetative growth and has proportionally long culms and big panicles with lots of grains in each panicle. No dwarf plant having the mutant trait was appeared among F₁ progenies from that cross, and F₂ generation segregated into dwarf and a few semi-dwarf plants, both of which showed mutant leaf-areal character, and tall plants with normal character. Although culms of these semi-dwarf plants were very short before heading, they elongated rapidly after heading, reaching as high as culm length of another cultivar Reimei.

Four Sho-Kei strains, G 26, G 29, G 31 and G 32, were selected from these semi-dwarf plants. All these strains are characterized by short, erect and deep-green flag leaves, and somewhat slender, elastic stems, thus resisting to lodging.

2) Photosynthetic activity of leaves of mutants

Experiments were conducted at the Tohoku National Agricultural Experiment Station, Morioka (Murakami et al., 1974). Upper three leaves including a flag leaf were cut off from the plants of the following strains or varieties immediately after heading. Strains or varieties tested were four semi-dwarf Sho-Kei strains,
G 26, G 29, G 31 and G 32, their parents, NF-1 and Meitoku 5, and two check varieties, Reimei and Sasanishiki. Experimental plants were grown by transplanting seedlings into paddy field. Rate of photosynthesis was measured on the cut leaves in the laboratory under 50 klux and atmospheric CO₂ concentrations at 30 °C, using a box (35×21×1 cm) made of acrylic resin. A flow of air in one hour was done at the rate of 70 to 150 l per unit of leaf area (dm²). Concentrations of CO₂ were determined with infrared gas analyzer.

A total of the upper three leaves of each semi-dwarf strain was 70 to 80% in length, 60 to 70% in breadth and 40 to 50% in area when compared with those

<table>
<thead>
<tr>
<th></th>
<th>Sasanishiki</th>
<th>Reimei</th>
<th>Meitoku 5</th>
<th>NF-1</th>
<th>G 26</th>
<th>G 29</th>
<th>G 31</th>
<th>G 32</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (g)</td>
<td>0.467</td>
<td>0.502</td>
<td>0.535</td>
<td>0.654</td>
<td>0.794</td>
<td>0.763</td>
<td>0.699</td>
<td>0.792</td>
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<tr>
<td>Total nitrogen (mg)</td>
<td>11.5</td>
<td>15.2</td>
<td>15.1</td>
<td>25.3</td>
<td>26.6</td>
<td>22.1</td>
<td>26.2</td>
<td>28.9</td>
</tr>
<tr>
<td>Protein-N (mg)</td>
<td>10.2</td>
<td>13.9</td>
<td>13.8</td>
<td>22.9</td>
<td>22.9</td>
<td>19.2</td>
<td>22.9</td>
<td>26.8</td>
</tr>
</tbody>
</table>

![Photosynthesis graph](image)

**Fig. 2.** Varietal differences in the rate of photosynthesis
- ■ photosynthesis rate per dm² leaf area
- □ photosynthesis rate per g leaf dry-matter
- ▲ photosynthesis rate per 10 mg leaf N
- ▶ photosynthesis rate per 10 mg leaf protein-N
of Sasanishiki. As shown in Table 1, however, dry-matter and the contents of total nitrogen or protein-nitrogen in the leaf blade of semi-dwarf strain were about twice as high as those of Sasanishiki. As demonstrated in Fig. 2, marked difference in photosynthesis was observed among the experimental materials. The semi-dwarf strains have a high rate of photosynthesis not only than Sasanishiki and Reimei, but also than their parents, NF-1 and Meitoku 5.

For the materials used in this experiment, the rate of photosynthesis had a close relation to their leaf blade dry-matter or nitrogen content. The highly significant correlation coefficients of the rate of photosynthesis (mg CO$_2$·h$^{-1}$) were obtained to be 0.9097** at 1% level with the dry-matter (g) and 0.9325*** at 0.1% level with the nitrogen content (mg N), respectively, per unit leaf area (dm$^{-2}$).

3) **Reaction of mutants to rice blast**

Because blast caused by *Pyricularia oryzae* is the most destructive of rice plant diseases, breeding stable resistant varieties is of the utmost importance. Studies were carried out on the reaction of the Sho-Kei semi-dwarf strains to the major races of blast fungus at the Shonai Branch Station, Yamagata Prefecture Agricultural Experimental Station. Nursery tests under natural infection with rice seedlings were done using three Sho-Kei strains, G 29, G 31 and G 32, their parents, Meitoku 5 and NF-1, and five check varieties, Ohu 244, Nakashin 120, Sasanishiki, Dewachikara and Fukunishiki. Predominant race was determined to be a Japan race C-1 by pathogenicity to a rice variety set which differentiates the races.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Symptom</th>
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<tbody>
<tr>
<td>Ohu 244</td>
<td>Most resistant</td>
</tr>
<tr>
<td>Nakashin 120</td>
<td>More susceptible</td>
</tr>
<tr>
<td>Sasanishiki</td>
<td>Most susceptible</td>
</tr>
<tr>
<td>Dewachikara</td>
<td>Most susceptible</td>
</tr>
<tr>
<td>Fukunishiki</td>
<td>Most resistant</td>
</tr>
<tr>
<td>Meitoku 5</td>
<td>More susceptible</td>
</tr>
<tr>
<td>NF-1</td>
<td>More susceptible</td>
</tr>
<tr>
<td>G 29</td>
<td>Most susceptible</td>
</tr>
<tr>
<td>G 31</td>
<td>Most resistant</td>
</tr>
<tr>
<td>G 32</td>
<td>Most susceptible</td>
</tr>
</tbody>
</table>

As shown in Table 2, NF-1 obtained from Fukunishiki by induced mutation was found to be more susceptible to Japanese C-1 race than its parent, Fukunishiki. It is noteworthy that reaction of semi-dwarf lines to rice blast varied markedly among them: the pathogenic races was no pathogenic to G 31, but pathogenic to G 29 and G 32. The result suggests, thus, that the upright, leaf-areal mutant
character was not bound up with the susceptibility to rice blast.

4) **Reaction of mutants to bacterial leaf blight**

Bacterial leaf blight (*Xanthomonas oryzae*) is one of the most damaging diseases of rice where heavy fertilizer supply is practiced. Studies on the resistance of rice varieties and strains of the pathogen have progressed considerably. Resistance of the semi-dwarf strains to bacterial leaf blight was tested by the needle-prick inoculation with virulent pathogens at the Shonai Branch Station, Yamagata Prefecture Agricultural Experiment Station. The susceptibility of rice strains and varieties was determined by the proportion of affected areas of flag leaves.

Three Sho-Kei strains, G 29, G 31 and G 32, their parents, Meitoku 5 and NF-1, and five check varieties, Ohu 244, Nakashin 120, Ootori, Sasanishiki and Fukunishiki, were tested for their resistance by the means of inoculation with 11 kinds of pathogenic isolates. Kuhara and his co-workers (1965) classified the bacterial isolates into three groups, I, II and III by inoculating to 10 varieties of rice. Therefore, the isolates tested could be classified into one of I, nine of II and one of III. All the materials were highly resistant to N5861 isolate of group I, and there was no differential reactions among the varieties and the strain. As well-known already, N5861 was less virulent isolate in Japan. Marked specific affinity between rice strains or varieties and bacterial isolates was observed when rice plants were inoculated with pathogens of group II. All strains and varieties were resistant to the isolates tested, such as H5809, OY6918 and OY7108. A mutant of Fukunishiki, NF-1, was found to be the most or more resistant to 4 isolates of group II, OY6914, OY6905, OY6920 and T7147, being highly resistant compared with its parent (Table 3). Resistances to two isolates, OY6902 and OY6908 of group II were not different markedly between NF-1 and its parent, Fukunishiki, and both were determined to be susceptible to these isolates. In general, Sho-Kei strains showed the similar resistance as one

<table>
<thead>
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<th>Varieties</th>
<th>I-N5861</th>
<th>II-OY6908</th>
<th>II-OY6920</th>
<th>II-OY7108</th>
<th>II-T7147</th>
<th>III-T7133</th>
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<tbody>
<tr>
<td>Ohu 244</td>
<td>R</td>
<td>—</td>
<td>R</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Nakashin 120</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ootori</td>
<td>R</td>
<td>MR</td>
<td>M</td>
<td>R</td>
<td>R</td>
<td>MR</td>
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<td>Sasanishiki</td>
<td>R</td>
<td>M</td>
<td>M</td>
<td>R</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Fukuniski</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>MR</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>NF-1</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>M</td>
<td>M</td>
<td>S</td>
</tr>
<tr>
<td>Meitoku 5</td>
<td>R</td>
<td>R</td>
<td>MR</td>
<td>R</td>
<td>R</td>
<td>M</td>
</tr>
<tr>
<td>G 29</td>
<td>R</td>
<td>MR</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>G 31</td>
<td>R</td>
<td>MR</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
</tr>
<tr>
<td>G 31</td>
<td>R</td>
<td>MR</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>MR</td>
</tr>
</tbody>
</table>

R: most resistant, MR: more resistant, M: more susceptible, S: most susceptible.
parent of them, Meitoku 5, to all the isolates tested. As demonstrated in Table 4, interestingly, it was found that the Sho-Kei semi-dwarf strains were the most or more resistant to an isolate T7133 of group III while each of their parent varieties was the most or more susceptible to it. From the results shown in Table 3, it might be concluded that the utilization of highly resistant rice varieties with upright, short and narrow leaves are possible to avoid damage due to bacterial leaf blight.

5) Reaction of mutants to insect pests

The important insects infesting rice plants in the Tohoku district of Japan are rice stem borer, Chilo suppressalis, and rice stem maggot, Chlorops oryzae. Studies were performed on the response of the Sho-Kei semi-dwarf strains to these insect pests at the Shonai Branch Station, Yamagata Prefecture Agricultural Experiment Station. The percentage of panicles injured by rice stem maggots was investigated under natural infestation. As shown in Table 4, NF-1 and G 32 were the most resistant, Sasanishiki, G 29 and G 31 more resistant, and Ohu 227, Fukunishiki and Meitoku 5 susceptible to the damage caused by rice stem maggots. All the strains having short and narrow leaves were less damaged than the varieties having normal leaves. This may be ascribed to less infesting dwarf or semi-dwarf mutants: for instance, NF-1 or each Sho-Kei strain escaped from the attack of rice stem maggots because of its dwarfness or semi-dwarfness.

Attacks of rice stem borers were made for by incubating 20 larvae just hatched out of the eggs into the sheath of the longest leaves of a potted rice plant. From the results shown in Table 5, it was determined that Meitoku 5 and NF-1 were the most resistant, Ohu 227 and G 29 more resistant, Sasanishiki and G 31 more susceptible, and Fukunishiki and G 32 the most susceptible to the attacks of rice stem borers. Seemingly, NF-1, a mutant of Fukunishiki, obtained a resistance to rice stem borers by induced mutations. Yet, because susceptible strains appeared among the progenies from the cross of NF-1 and Meitoku 5, the resistance to rice stem borers
Table 5. Reaction of rice varieties to rice stem borer

<table>
<thead>
<tr>
<th>Varieties</th>
<th>No. of analyzed culms</th>
<th>% of injured culms</th>
<th>No. of survived larvae</th>
<th>% of survived larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sasanishiki</td>
<td>55</td>
<td>10.1</td>
<td>9</td>
<td>15.0</td>
</tr>
<tr>
<td>Oho 227</td>
<td>55</td>
<td>5.5</td>
<td>7</td>
<td>11.7</td>
</tr>
<tr>
<td>Fukunishiki</td>
<td>44</td>
<td>9.1</td>
<td>17</td>
<td>28.3</td>
</tr>
<tr>
<td>NF-1</td>
<td>59</td>
<td>1.7</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>Meitoku 5</td>
<td>48</td>
<td>2.1</td>
<td>1</td>
<td>1.7</td>
</tr>
<tr>
<td>G 29</td>
<td>55</td>
<td>1.8</td>
<td>8</td>
<td>13.3</td>
</tr>
<tr>
<td>G 31</td>
<td>41</td>
<td>7.3</td>
<td>11</td>
<td>18.3</td>
</tr>
<tr>
<td>G 32</td>
<td>39</td>
<td>7.7</td>
<td>15</td>
<td>25.0</td>
</tr>
</tbody>
</table>

was thought to be induced with a separate mutation from that of short and narrow leaves.

6) Susceptibility of mutants to cool-summer damage

As testing methods for resistance to cool-summer damage, field tests with irrigation of cool water for a long term and plant tests under cool condition using a growth cabinet were carried out at Fujisaka Branch Station, Aomori Prefecture Agricultural Experiment Station. The former tests result in cool-summer damage due to delayed growth, while the latter tests due to floral impotency.

Firstly, cool water, about 17 °C at water inlet, was irrigated 6 to 8 cm deep for 41 days (10 July to 20 August) into the experimental paddy field. From each strain or variety 10 panicles of the longest culm of a plant were harvested and the

![Sterility Chart]

Fig. 3. Resistance of rice varieties to cool-summer damage
- ■ sterility due to delayed growth
- ■ sterility due to floral impotency
seed setting rate was investigated on these panicles. As demonstrated in Fig. 3, a dwarf NF-1 and two semi-dwarf G 31 and G 32 had much more sterile grains when grown at the paddy field irrigated with cool water, and then they were determined to be the most susceptible to cool-summer damage due to delayed growth. It is thought that NF-1 and Sho-Kei semi-dwarf strains are late-maturing and nanism during their vegetative growth so that their shoot apices are vulnerable to injuries caused by cool irrigation water.

Secondly, plants at booting stage were transferred into the growth cabinet, and kept for 5 days at 15 °C. Resultant decrease of seed setting was investigated on the panicles. Fig. 3 indicates that most of the varieties are susceptible to cool temperature treatment. A Sho-Kei G 31 strain differed somewhat from its relatives, Fukunishiki, NF-1, Meitoku 5 and G 32 to be resistant to damage caused by cool temperature treatment: its resistance was comparable to those of Fujiminori, Reimei and Shimokita which have been known as the cool tolerant varieties.

7) Response of mutants to inundation

As plants of the Sho-Kei semi-dwarf strains are pygmy at vegetative growth stage prior to their panicle emergence, it is afraid that they are vulnerable to damage caused by overhead flooding. Such flood damage will be accelerated by shading of sunlight due to submergence with mud water. Using the Sho-Kei semi-dwarf strains, G 29, G 31 and G 32, with three check varieties, Hayanishiki, Kiyonishiki and Sasanishiki, their response to overhead flooding was tested at 10 cm water depth from tip of leaves at the Faculty of Agriculture, Yamagata University. Shading of sunlight was carried out by screening with black cheese cloth, so that the light

<table>
<thead>
<tr>
<th>Varieties</th>
<th>No flooding and no shading</th>
<th>No flooding and shading</th>
<th>Flooding and no shading</th>
<th>Flooding and shading</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 1st experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hayanishiki</td>
<td>22</td>
<td>23</td>
<td>117</td>
<td>113</td>
</tr>
<tr>
<td>Kiyonishiki</td>
<td>21</td>
<td>23</td>
<td>143</td>
<td>138</td>
</tr>
<tr>
<td>Sasanishiki</td>
<td>19</td>
<td>19</td>
<td>164</td>
<td>163</td>
</tr>
<tr>
<td>G 29</td>
<td>25</td>
<td>23</td>
<td>203</td>
<td>213</td>
</tr>
<tr>
<td>G 31</td>
<td>19</td>
<td>16</td>
<td>171</td>
<td>195</td>
</tr>
<tr>
<td>G 32</td>
<td>21</td>
<td>11</td>
<td>162</td>
<td>171</td>
</tr>
<tr>
<td>2) 2nd experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiyonishiki</td>
<td>9</td>
<td>9</td>
<td>32</td>
<td>26</td>
</tr>
<tr>
<td>Sasanishiki</td>
<td>3</td>
<td>16</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>G 29</td>
<td>16</td>
<td>22</td>
<td>46</td>
<td>41</td>
</tr>
<tr>
<td>G 31</td>
<td>14</td>
<td>30</td>
<td>39</td>
<td>37</td>
</tr>
</tbody>
</table>

Figures show seedling growth during treatment (%).
intensity reduced to $62 \pm 18.3\%$. Treatments of submergence using four-leaves seedlings were given for a week in deep-water of $23.0 \pm 1.8 ^\circ C$ in the 1st experiment and for 5 days in $18.2 \pm 0.8 ^\circ C$ in the 2nd experiment, respectively. Table 6 shows the elongation rate of the tested seedlings during submergence treatment. Seedlings of the Sho-Kei semi-dwarf strains elongated more during overhead flooding and showed less response to shading treatment, compared with those of the check varieties. Thus, it is expected that the Sho-Kei semi-dwarf strains are resistant to flood damage.

The Sho-Kei mutant strains had the same number of primary panicle-branches and kernels produced on them if compared with commercially grown varieties. However, they have a defect of extremely small number of secondary panicle-brances (Yamaguchi and Watanabe, 1977). Current studies are concerned with the improvement of this defect.

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イネの部分不対合および細葉突然変異の放射線
生物学的なならびに育種学的研究

山口 彦之
東京大学農学部放射線遺伝学科教室
渡辺昌幸・佐藤良一・上林健徳
山形県農業試験場庄内支場

突然変異はメンデルの法則の再発見と同時に知られた現象であり、遺伝学は突然変異体をうまく利用して発展してきたといえる。ここでは2つの研究をとりあげた。

1) 部分不対合突然変異体による突然変異損傷の修復の研究

イネ種子のG1期にガンマ線照射あるいはEMS処理をしたとき突然変異をほとんど誘起しなかった。このことは前突然変異損傷の修復が効率よくなされていることを示唆した。高等生物の遺伝子組換えは染色体複製後に起こるもので、この修復は1種の組換え修復と推定される。この組換え修復が起こるための必要条件は、体細胞における相同染色体の近接配置である。このような近接配置がオオムギやライムギで明らかにされ、Thomas (1973) はエンパクの不対合系統がその体細胞においても相同染色体の近接程度を低くしていることを見いだしている。したがって、部分不対合系統が高い放射線感受性を示すかどうかを検討した。

部分不対合ホモ型および正常型の乾燥種子にガンマ線を照射したところ、生残率、種子総実率の低下ならびに突然変異率の増加は正常型よりも部分不対合型で顕著であった。こうして相同染色体間の組換えによる修復が示唆されたが、この修復はエラーを伴わない完全型であることが予想される。

2) 細葉突然変異体の育種学的研究

集約栽培下の多収性は葉が直立的であること、葉幅が狭いことと密接に関係していることから指摘されているので、そのような草型の突然変異をえて、その特性について検討することとした。

水稻品種フクシキの全生育期間乾燥焼用によって、矮性で葉幅が極めて狭く、かつ葉身の短い突然変異体NF-1をえた。このNF-1に普通の葉幅をもつ明徳5号を交配し、出穂後に稈が長く伸びる半矮性の細葉系統を育成した。

まず、これら細葉系統の光合成特性を調査した。単位葉面積あたり葉身乾物重、同窒素含量、同光合成速度はいずれもササニシキの1.5〜2倍と大きかった。さらに細葉系統の諸特性のうち特異検体試験の対象形質について検討した。葉もち病抵抗性は系統間差
異が認められ、葉幅と抵抗性との間には関係がないと思われた。白葉枯病抵抗性はいろいろな菌系で検定したが、これも葉幅の広狭と関連しなかった。一般に細葉系統は普通型品種よりもカラバエ耐性が強かったが、短種による回避性も示さなかった。NF-1 の二化メイ虫耐虫性は原品種のフランシネより強かったが、葉幅と耐虫性との間に関係はないものと考られる。長期冷凍接種による遅延型冷害に対する抵抗性は細葉系統が著しく低下していた。これは矮性、半矮性のため生長点がつねに冷水中にあることによると思われる。細葉系統は冠水、遮光の光に対しては抵抗性であるらしい。

質疑応答

松尾：DNA の組換え修復のメカニズムについてどう考えられるか説明してほしい。

山口：微生物で知られている組換え修復機構は DNA 複製で録 DNA を作り、それの悪いところ（害を受けたところ）を一方の DNA に集め、それを犠牲にして生き残るというメカニズムである。だが修復酵素については切り出し修復などにはメカニズムは明らかでない。微生物では 2n でなく n ののでこのようにして組換え修復が生ずるが、高等生物では 2n のので微生物で知られる組換え修復以外に染色体レベルの組換え修復のメカニズムがあるのではないか。こうして高等生物では DNA の切り出し修復といわゆる微生物でいう組換え修復、さらに高等生物特有の染色体レベルでの組換え修復の三つがあると考えられる。染色体レベルの組換えの修復は放牧研の中井氏らが酵母で見つかっており、そういう可能性を中井氏らと私が指摘している。

神山：パーシャルアンナプラスス系統と正常系統のそれぞれに現われた突然変異の型の内訳について説明してほしい。つまりパーシャルアンナプラスス型においては点突然変異と欠失型突然変異のどちらが多いかということ、もう一つは微生物においては組換え修復の変異体は点突然変異および欠失型突然変異のいずれにおいても放射線により誘発されないといわれている点との関連において、このパーシャルアンナプラスス系統において誘発される突然変異の誘発機構についてどう考えているか。

山口：正常系統の突然変異頻度は照射線量とともに直線的に増加するから点突然変異がほとんどであり、パーシャルアンナプラスス系統の場合は 2 ヒット型だから点突然変異と小さな欠失と混っていると考えられる。このちがいは体細胞組換えがあるかないかの差だから多分染色体レベルの組換え修復は小さな欠失をならす修復であろうと考えられ、そういう意味でいわゆる微生物の組換え修復とはちがっている。

山下：このパーシャルアンナプラスス突然変異は比較に用いた金南風と同じような関係にあるのか、部分不稔の種子稔実率はどれ位か。

山口：パーシャルアンナプラスス系統の K648 は九州大学からもらったものであるが、親がわからないので正常系統として金南風を使用した。だからバックグラウンドはちがう。放射線感受性の差異はパーシャルアンナプラススのためであろうと推定している。それからこの実験をさらに継続しなかったのはつねに不稔でパーシャルアンナプラスス系統を維持するのが大変であることと、部分不稔系統が感受性が高いので、多くの数を取扱わねばなら
ばならない。特に突然変異の実験では大変な労力がかかるためである。パーシャルアシンナプス系の種子不稔率は大体70%ぐらいである。

鯖鋼：この実験で使われた系はアシンナプスであるが、対合はするが交配に至らない。即ちアシンナプス系でも同様に体細胞で相同染色体の近接がないと期待できる。もう一つはRILEY & MILLER（1966）が大麦のアシンナプス系は2ヒット型染色体異常で多数の感度性が高いことを報告している。もしもそうならばM_r総性も当然低くなると考えられるか実験結果との関連をどう考えるか。

山口：この実験のアシンナプス系と異なり、アシンナプスは相同染色体が最初近接して早く分離すると考えられる。アシンナプスには互いに矛盾した報告があってアシンナプスは放射線感受性を高めるというデータと高めないというデータの両方がある。多分アシンナプス系では相同染色体が最初近接している時に組換え修復をやるから放射線感受性の増大は関係しないのではないかと考えている。

松尾：細葉突然変異体の個体当たり、または単位面積当たりの乾物生産量はどうなっているかデータがあったら聞きたい。

山口：普通栽培の収量はササニシキに比べて劣るが、一つの欠点は二次枝梗の石数が少ないということである。今この二次枝梗の改良のために実験を行っているが、超密植の時はササニシキに比べて収量がよくなる。特に超密植（24kg/a）の直接栽培ではササニシキよりかなり収量がある。

藤井：細葉突然変異体は病害虫抵抗性、冷害抵抗性など多くの形質に関しても変異を示している。これが遺伝子の多面的発現ならば育種に大きな意味を持つ、このような変異は多面発現と考えてよいか。

山口：一応稔性が低下していないという事で点突然変異型の突然変異と思うが、ここで調べた形質がすべて環境や病害虫との相互作用であり、特にこの突然変異体は葉の形が変っていることで普通のイネよりも感気象的にはかなり変化していると思われ、厳密な意味で多面的発現と考えてよいかどうかわからない。

木下：イネでは自然突然変異として、細葉および捲葉なる名称で種々の葉幅の変異がみられる。研究に用いた細葉はどの程度の葉幅の減少であるか。

山口：葉幅はササニシキの60〜70%で、葉が多少捲いている。

米沢：超密植条件では細葉型突然変異系の単位面積当たり収量が通常型品種に比べて相対的に増加するという話であったが、収量の絶対値そのもの増加するのか。

山口：ササニシキは密植すればするほど、収量は下っていくが、細葉系統の方は密植するとだんだん収量が上っていく傾向にある。
PHYLOGENIC DIFFERENTIATION IN FATTY ACID BIOSYNTHESIS AND MUTATION

Shigesaburo Tsunoda and Priya R. Kumar

Faculty of Agriculture, Tohoku University, Sendai, Japan

In recent years there has been extensive research on the genetic control of fatty acid components of edible oils like rapeseed (Harvey and Downey 1964, Dorrell and Downey 1964, Morice 1972, Jönsson 1973, Röbbelen and Thies 1973, Lee et al. 1974b, Kondra and Thomas 1975), soybean (White et al. 1961), sufflower (Knowles and Hill 1964), linseed (Yermanos and Knowles 1962, Badwal and Ahuja 1976) and groundnut (Tai et al. 1972 as referred by Tai and Young 1975). Compared to other oil crops, our understanding on the chem-genetical aspects in rapeseed has increased enormously. This is partly because of the influence of dietary rapeseed oil on the development of atherosclerosis, hypercholesterolemia, lipidosis, necrotic lesions and histopathological distortions (Carroll 1953, Kinsell and Sinclair 1957, Roine et al. 1960, Abdelatif and Vles 1970, 1973, Beare-Rogers 1970, 1976, Rocquelin et al. 1973, Walkar 1976), and partly due to its wide range of utilization in various industries.

In view of the increasing importance of rapeseed in the world trade, plant breeding programme for the improvement of oil quality in rapeseed was initiated in Canada in 1958 by Downey and his colleagues, followed by Lööf and Appelqvist in Sweden, Röbbelen and his colleagues in Germany, Morice in France and Krzymanski in Poland. In Japan, similar studies were undertaken by Shiga and his group in 1968 with Japanese and Korean varieties of Brassica napus. With Brassica campestris, we initiated a quality breeding project in 1975 so as to improve the oil and the seed meal quality of the Indian rape, Brown Sarson. Besides, an experiment was also conducted on the evaluation of fatty acid composition of a wide array of wild and cultivated species of the family Cruciferae* in order to search for "new" oilseed crops with favourable lipid composition for edible (Kumar and Tsunoda 1976) and non-edible purposes.

*1 Visiting Scholar; Permanent address: Department of Genetics Haryana Agricultural University, HISSAR-125004, INDIA
*2 Collected by the senior author from the west Mediterranean region in 1975 during the Plant Exploration of Brassica and allied genera.
In the present review, an attempt is made to study the relationship between the phylogeny and the differentiation of fatty acid biosynthesis. Further, the gene analysis of fatty acid biosynthesis and mutations are discussed with particular reference to the cruciferous oilseed.

I Relationship between the phylogeny and differentiation of fatty acid biosynthesis

I-a Evolution of fatty acid biosynthesis from lower organisms to higher plants

Wagner and Pohl (1966), as referred by Röbbelen (1975), made a survey of the fatty acid composition over a wide range of organisms. As is evident from Fig. 1,

Fig. 1. Evolution of fatty acid synthesis (after Wagner and Pohl 1966)

an evolutionary arrangement reveals that the number of saturated fatty acids possible within the plant kingdom was almost complete in the Bacteriophyta, while the ability to synthesize unsaturated fatty acids evolved more slowly. Thus, bacteria form only monoenoic acids (up to C18: 1) by means of an anaerobic process. In higher systematic groups additional aerobic system of fatty acid biosynthesis originated. Apparently the synthesis of polyenoic fatty acids is bound to chlorophyll, oxygen and light. Soon after the synthesis of polyenoic acids originated, the evolution of the biogenesis of fatty acid quickly proceeded to its maximum in the green
algae with C22: 6. With transition of plants from water to land, abilities of synthesizing the long chain fatty acids and polyenoic acids were reduced to some extent, apparently in consequence of ecological needs. The synthesis of eicosenoic and erucic acids was stopped in many plants and also highly unsaturated types disappeared (e.g., all of the C16 polyenoics), leaving in existence the α-linolenic acid as the highest unsaturated form of Spermatophyta.

When we examine the fatty acid composition in fishes and mammals, we find that they differ greatly from each other. While, fishes contain oil rich in polyenoic fatty acids, mammals are mostly composed of saturated and monoenoic fatty acids.

1-b Differentiation of angiosperms in relation to fatty acid composition

Thies (1968) demonstrated that the various oil crops exhibit five basic types.
in the pattern of fatty acid formation (Fig. 2). *Brassica*, compared to other oil crops like linseed, safflower, groundnut and cocos, is found to be different in their ability to synthesize eicosenoic and erucic acids. Further, *Brassica* can also synthesize higher amount of polyenoic acids. Based on these studies, *Brassica* group can be said to be of the primitive type as it still maintains the ability of synthesizing the long chain fatty acids (C20: 1, C22: 1) and high concentration of unsaturated fatty acids. The other major oil crops, on the other hand, lost their ability to

Fig. 3. Distribution of the major fatty acids and oil content in 54 wild species of Cruciferae (P.R. Kumar and S. Tsunoda, unpublished)
synthesize the long chain fatty acids. Besides, some oil crops like groundnut, sunflower and safflower even lost their ability of synthesizing trienoic acid (C18:3).

Taking into account the aforesaid facts, we tried to find the relation between the phylogeny of angiosperms and their fatty acid composition on the basis of the phylogenetic links, as postulated by Takhtajan (1954). But we failed to find out any definite relation. The observed differences in the fatty acid composition appear to be due to some physio-ecological causes, rather than the phylogenetic.

I-c Fatty acid composition in relation to the phylogeny of Cruciferae

The fatty acid composition of cultivated and wild species of Cruciferae has been evaluated by a number of workers. Both in cultivated (Craig and Wetter 1959, Craig 1961, Downey 1963, Appelqvist 1970, Lee et al. 1974a) and wild (Mikolajczak et al. 1961, Miller et al. 1965, Goering et al. 1965, Appelqvist 1971, P.R. Kumar and S. Tsunoda, unpublished) species, substantial variability in C18 and C22 were recorded (Fig. 3).

According to Prantl's (1890) system of classification, the family Cruciferae is divided into four tribes, viz., Sinapeae, Thelypodieae, Hesperideae and Schizopetaleae. Miller et al., (1965) while trying to find the relationship between Prantl's tribe and fatty acid composition, observed that oil containing the highest concentration of erucic acid were in tribe Sinapeae, while those possessing less than 2% erucic acid, belonged to tribe Schizopetaleae. We also tried to find out a possible relationship of fatty acid spectrum to the phylogeny of cruciferous plants based on Schulz's (1936) system with data of different workers (Mikolajczak et al. 1961, Miller et al. 1965).

![Diagram of Cruciferae phylogeny](image)

Fig. 4. Diagrammatic representation of the relations between the different tribes of Crucifere (Schulz 1936)
1965, Appelqvist 1971) and our new observations. As for the erucic acid concentration, species belonging to the tribe Brassiceae showed higher values, while some of the species of tribe Alyssaeae, Arabideae, Matthioleae, Hesperideae, Sisymbrieae, Lepidieae and Schizopetalaeae showed zero or very low concentration of erucic acid. In the Schulz’s system, such tribes which include species having zero or low level of erucic acid are positioned at the top of the phylogenetic tree (Fig. 4).

With regard to the linolenic acid, none of the species of Cruciferae examined so far, are free from linolenic acid. However, two wild species, Conringia orientalis belonging to tribe Brassiceae (sub-tribe Moricandiinae) and Nasturtium officinale of tribe Arabideae produce oil having very low concentration, 3.7 and 1.7% of linolenic acid, respectively (P.R. Kumar and S. Tsunoda, unpublished). Similar low values of linolenic acid have been reported earlier by Miller et al. (1961) for Nasturtium officinale.

I-d Variation within genus and species

So far the interspecific variation is concerned, Crambe abyssinica and Crambe maritima produce oil containing erucic acid ranging from 55.2 to 59% (Mikolajczak et al. 1961, Goering et al. 1965) and 26.3 to 32.6% (Appelqvist 1971), respectively. In Crambe tatarica, Miller et al. (1965) reported 27% erucic acid. Likewise, the interspecific variation within Malcolmia, Matthiola, and Sinapis are also observed. Miller et al. (1965) in Malcolmia africana and Malcolmia cabulica and Kumar and Tsunoda (1976) in Malcolmia ramosissima reported zero erucic acid. On the other hand, Mikolajczak et al. (1961) in Malcolmia maritima reported 18% erucic acid. Similarly in Matthiola incana, Joshi and Bhakuni (1959) as referred by Mikolajczak et al. (1961) reported high erucic acid, while Matthiola bicornis (Mikolajczak et al. 1961) and Matthiola parviflora (Kumar and Tsunoda 1976) were found to be free from erucic acid. In two species of Sinapis, viz., Sinapis alba and Sinapis arvensis, Miller et al. reported 51 and 35% erucic acid, and P.R. Kumar and S. Tsunoda (unpublished) recorded 54.7 and 33% erucic acid, respectively.

As for examples of variation within the species, we will consider Brassica napus and B. campestris among the cultivated species and Torularia torulosa among the wild species. Evaluating the fatty acid composition of 51 varieties of B. napus and 28 varieties of B. campestris, Downey (1963) recorded concentration of erucic acid ranging from 28 (German forage variety Liho) to 53% (variety Hamburg) of B. napus, and 62 to 17% in Indian rape, yellow Sarson and the variety Polish of B. campestris. Taking the advantage of such a large variation, Downey and his colleagues made single plant selections for low erucic acid concentrations within varieties Liho and Polish. Their intensive research finally led in release of varieties like Oro, Zephyr, Midas and Tower of B. napus and Torch of B. campestris (Anon
PHYLOGENIC DIFFERENTIATION IN FATTY ACID BIOSYNTHESIS

1974). Similar studies in Germany, Sweden and France have resulted in release of rapeseed varieties free from erucic acid, *viz.*, Erglu and Kosa in West Germany; Erra and Expander in East Germany; Linus and Brink in Sweden and Primor in France. These varieties are widely cultivated in the respective country in place of earlier varieties possessing 40—50% erucic acid (Röbbelen and Nitsch 1975).

In case of wild species of Cruciferae, Miller *et al.* (1965) recorded 25% erucic acid in *Torularia torulosa*, while P.R. Kumar and S. Tsunoda (unpublished) observed a strain of this species producing oil free from erucic acid. Further studies with wild and cultivated species showing the favourable lipid composition are in progress in order to assess the potential of these species as “new” oilseed crops.

II Gene analysis of fatty acid biosynthesis and mutation

II-a Length of carbon chain

A knowledge about the inheritance of the long chain fatty acids (C20: 1, C22: 1) is essential while breeding for oil quality of rapeseed. Studies on the mode of inheritance of erucic acid in *B. campestris* revealed that the synthesis of erucic acid is controlled by a single major gene, exhibiting no dominance and acting in an additive manner (Dorrell and Downey 1964). In this species, three alleles, *E*^b^, *E*^c^ and *e* contributing 15%, 30% and zero, were involved in establishing the erucic acid level (Downey 1966). In *B. napus*, on the other hand, two gene pairs displaying no dominance and acting in an additive manner controlled the synthesis of erucic acid (Harvey and Downey 1964), and each *E*^a^ contributing about 10% erucic acid to the seed oil (Downey and Dorrell 1971, Table 1). The presence of two gene pairs in *B. napus* appears to be due to its amphidiploid nature containing the genomes of *B. oleracea* and *B. campestris* having at least one gene for erucic acid biosynthesis, respectively. Krzymanski and Downey (1969) demonstrated the presence of another allele, *E*^d^ in *B. napus* which contributes about 3.5% (5% in a review by Downey and Dorrell 1971) erucic acid. This allelic series makes possible the fixing of the erucic acid concentration at almost any desired level up to 60% of the total fatty acids. While trying to develop varieties of rapeseed with higher concentration of erucic acid for various industrial purposes, Appelqvist (1969) analysed the interseed variation in some species of the sub-tribe Brassicinae with a high average erucic acid concentration and demonstrated a theoretical upper limit of ca. 65% for erucic acid and other long chain fatty acids in *B. napus*, *B. campestris* and *Crambe abyssinica*. Such results in conjunction with data on erucic acid positioning in the triglyceride molecule suggest that the Cruciferae lack the capacity to insert erucic acid at the central position of the triglycerides (Appelqvist 1969).
Table 1. Proposed genotypes and percent erucic acid in *B. napus* and *B. campestris* (Downey and Dorrell 1971)

<table>
<thead>
<tr>
<th>Erucic, %</th>
<th>Genotype</th>
<th>Genotype</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>eee</td>
<td>ee</td>
<td>ee</td>
</tr>
<tr>
<td>5</td>
<td>Ee</td>
<td></td>
<td>Ee</td>
</tr>
<tr>
<td>10</td>
<td>Eeee</td>
<td>EE</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td>Ee</td>
</tr>
<tr>
<td>20</td>
<td>EEee</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>EEEe</td>
<td>EE</td>
<td>Ee</td>
</tr>
<tr>
<td>35</td>
<td>EEEE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>50</td>
<td></td>
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<td>55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td>EE</td>
</tr>
</tbody>
</table>

II-b Degree of desaturation

From the food technology standpoint, higher concentrations of monoenoic (C18:1) and dienoic (C18:2) fatty acids are favourable, while the trienoic (C18:3) fatty acid is unfavourable because its three double bonds sensitive to oxidation leads to a bad, “green” taste of the product and to other dietary disadvantages. It is, therefore, desirable to decrease, or if possible eliminate the linolenic acid and increase the levels of oleic and linoleic acids in the rapeseed oil.

In the desaturation chain (Fig. 5), at least two independent enzymes or system of enzymes seem to be operating for the synthesis of linoleic and linolenic acids,
respectively (Thies 1968, Appelqvist 1968, and Jönsson 1975). In safflower, Knowles and Hill (1964) conducted the genetic analysis with C18 components of fatty acid and observed that a single dominant locus with three alleles OL, oL1, oL controlled the levels of oleic and linoleic acids. By an efficient management of genes with small effects Knowles (1972), as referred by Knowles (1975), was able to increase the concentration of oleic acid to the extent of 85%. In rapeseed, however, the desired changes, i.e., the decrease in C18:3 and the increase of C18:1 and C18:2, especially of the latter, has been difficult. Even in wide screening processes, as already mentioned, Mikolajczak et al. (1961), Thies (1968), Appelqvist (1971), Röbbelen (1972) and recently P.R. Kumar and S. Tsunoda (unpublished, Fig. 3) did not find any variety or species of the family Cruciferae having oil free from linolenic acid. This is rather surprising, since the higher concentration of linoleic, and zero linolenic acids are quite common in other edible oils like safflower, cottonseed, corn etc. (Godin and Spensley 1971). Several assumptions have been put forward for the failure of getting oil free from linolenic acid. One of the assumptions is the relationship between the photosynthesis and desaturation chain of fatty acids. While studying the mutant lines having high and low linolenic acid contents in rapeseed, Thies (1970, 1971) observed a positive correlation between the higher concentration of linolenic acid and chlorophyll content in maturing seeds. Thies (1971) further pointed out that whenever the seed oil is formed within a green embryo, as in rapeseed and soybean, linolenic acid appears exclusively. Such findings support the idea that linolenic acid is not only an essential constituent of the green chloroplast membranes, but that photosynthetic potentials put forward the desaturation of fatty acid (Röbbelen 1975). However, Rakow and McGregor (1975) observed no clear relationship between the pattern of fatty acid and the seed chlorophyll content in rapeseed. Studies with soybean seeds have also failed to show a relationship between the chlorophyll content and linolenic acid concentration (Fehr et al. 1971).

In view of the absence of linolenic acid free genotypes in naturally existing germplasm of Cruciferae, Röbbelen and Rakow (1970) in Germany and Morice (1975) in France started mutation experiments in B. napus with Canadian variety “Oro” and French variety “Primor,” respectively. Their objective was to generate variability in the population and identify genotypes having the desired fatty acid characteristics, viz., low linolenic acid (<1%) and high linoleic acid (~50%) in the seed oil of zero erucic genotype. Using physical and chemical mutagens Rakow (1972) did not get any linolenic acid free genotype. However, he isolated a mutant (M57) containing a lower amount (5.6%) of linolenic acid, but its linoleic concentration remained unchanged (Table 2, group I). After getting success in this experi-
Table 2. Contents of polyenoic fatty acids and morphological description of some Canadian German "zero erucic" spring rapeseed cultivars (group O; 'Egra' and 'Tower' are low also in glucosinolates), of earlier mutants selected in Goettingen after mutagenic treatment of 'Oro' (groups I and II), and of new mutants obtained from the 'Oro'-mutant M 57 (group III) (Röbbelen and Nitsch 1975)

<table>
<thead>
<tr>
<th>Mutant group</th>
<th>Genotype</th>
<th>Fatty acids (percent)</th>
<th>Plant height (cm)</th>
<th>Silique length (cm)</th>
<th>Fertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C 18:2</td>
<td>C 18:3</td>
<td>C 18:3/C 18:2</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Tower</td>
<td>22.80</td>
<td>9.65</td>
<td>0.42</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>Oro</td>
<td>21.50</td>
<td>9.77</td>
<td>0.45</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Egra</td>
<td>20.32</td>
<td>10.14</td>
<td>0.50</td>
<td>160</td>
</tr>
<tr>
<td>I</td>
<td>M 57</td>
<td>22.40</td>
<td>5.60</td>
<td>0.25</td>
<td>110</td>
</tr>
<tr>
<td>II</td>
<td>M 3</td>
<td>32.53</td>
<td>7.39</td>
<td>0.22</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>M 6</td>
<td>23.45</td>
<td>3.51</td>
<td>0.15</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>M 8</td>
<td>33.77</td>
<td>8.26</td>
<td>0.24</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>M 11</td>
<td>37.92</td>
<td>8.42</td>
<td>0.22</td>
<td>70</td>
</tr>
<tr>
<td>III</td>
<td>M 40</td>
<td>30.50</td>
<td>5.36</td>
<td>0.17</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>M 41</td>
<td>26.13</td>
<td>3.81</td>
<td>0.15</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>M 42</td>
<td>24.59</td>
<td>3.50</td>
<td>0.15</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>M 43</td>
<td>27.69</td>
<td>3.31</td>
<td>0.12</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>M 44</td>
<td>29.07</td>
<td>4.48</td>
<td>0.15</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>M 45</td>
<td>28.27</td>
<td>3.97</td>
<td>0.14</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>M 46</td>
<td>32.38</td>
<td>4.50</td>
<td>0.14</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>M 47</td>
<td>30.06</td>
<td>3.24</td>
<td>0.11</td>
<td>70</td>
</tr>
</tbody>
</table>

In the M₄ generation, they isolated the eight best mutants (M40—M47) which showed still lower concentrations of linolenic acid and higher concentrations of linoleic acid (Table 2, group III). However, their growth behaviour and fertility were not good. A comparison of the single mutants in Table 2, revealed that M47 has the linoleic acid concentration 34% higher than the parent M57, and linolenic acid is reduced by 42% resulting in very low quotient of 0.11. Based on these findings, Röbbelen and Nitsch (1975) suggested the possibility of selecting recombinants with further improved polyenoic acids composition and the better growth behaviour after crossing of complementary mutants.

**Conclusion**

This symposium is held for discussing the usefulness of induced mutation in genetical, physiological and ecological studies of crop plants. The mutation
experiments for oil quality of rapeseed were initiated rather recently, as reviewed above, mainly from the viewpoint of practical breeding to get zero or nearly zero linolenic acid genotypes. The important problem to be solved is the difficulty of combining the high yielding growth behaviour and the desired oil quality. As stated by Röbbelen (1975), detailed comparative studies of the physiological and biosynthetic mechanisms underlying biological processes based on the diverse natural and induced genetic variants may provide us the key to overcome the problem.

References


脂肪酸生合成についての系統分化と突然変異

角田重三郎・Priya R. Kumar

東北大学農学部

植物油、特にナタネ油の品質改良に関連して、脂肪酸組成の遺伝的支配についての研究が進行している。特にカナダのDowneyら、スウェーデンのAppelqvistら、ドイツのRöbbelenら各派の研究は活発である。日本では、平塚などで志賀氏を中心とする研究グループが日本と韓国の*napus*系ナタネの改良を目指し、私共はインドの*campestris*系ナタネの改良を目指し、この方面の研究にとりくんでいる。ここでは、Ⅰ．植物の系統発生と脂肪酸生合成、Ⅱ．脂肪酸生合成についての遺伝子分析と突然変異について、最近までの研究をとりまとめてみたい。

Ⅰ．植物の系統発生と脂肪酸生合成

Wagner & Pohl (1960―Fig. 1) はバクテリアから種子植物にいたる広範な材料について、脂肪酸生合成の進化をたどっている。不飽和度の高い脂肪酸の形成はクロロフィル、光、酸素と関連があるようで、緑藻はC22:6を合成する。植物の陸生化と伴ない、長鎖の脂肪酸、不飽和度の高い脂肪酸の形成が低下了した傾向が見られる。Thies (1968―Fig. 2)は油料作物を油の脂肪酸組成より5疎に分類しているが、アブラナ型を除く他の型では、C20:1、C22:1などの長鎖の脂肪酸を形成せず、またアブラナ型およびアマ型を除く他の型ではC18:3を形成せず、より飽和したC18:1あるいはC18:2を蓄積する。アブラナ型は「原始型」とも言える。アブラナ科植物の種子の多くはThiesのアブラナ型の脂肪酸形成を行なうが、科内で相当の変異が見られる。筆者らは地中海地域の野生種54種について脂肪酸組成を観察し(Fig. 3)、他の研究者の報告をと合わせて考察を加え、離離ルシウシの種子油をもつ植物がSchulz (1936)の系統樹(Fig. 4)の上位に位置する達(tribes)に属する傾向を認めた。脂肪酸組成についての変異は、同一達内、属内、種内でも認められる。さらに同一品種の個体間でも認められる。Downeyらは*napus*系および*campestris*系ナタネの種子油のエリシウシ酸含量の品種間差を調査し、低含量の*napus*系品種のライホ、*campestris*系品種のポーリッシュについて更に個体選抜を行なうと、エリシウシ酸の系統を作出することに成功した。この成果を基にして、カナダ、スウェーデン、ドイツ、フランスでエリシウシ酸のナタネ品種が育成されている。ノレイン酸(C18:3)については、筆者らの調査をふくめ、アブラナ科の植物のうち、ノレイン酸をふくまない種子油をつくる植物は見出されていない。Thies (1971)は、ナタネやアマイのように緑色の胚中で種子油が形成される場合には、例外なくノレイン酸が形成されるとしている。しかし、アブラナ科中でも、比較的ノレイン酸含量の少ない種子油をつくる種も見られる(Fig. 3)。
II. 脂肪酸生合成についての遺伝子分析と突然変異

エルシン酸合成は Brassica campestris では、1 遺伝子座の相加的に働き、対立遺伝子系により、B. napus では2遺伝子座の相加的に働き、対立遺伝子系により支配されている。対立遺伝子の組合せにより、全脂肪酸のうちエルシン酸含量が零から 60％ 程度となる。アブラナ亜属の種を通じて最高値 65％ で、これは triglyceride の中央の位置にはエルシン酸が着かない事と符合している (Appelqvist 1969)。

Röbbelen らは、人為突然変異により低リノレイン酸の種子油を形成するナタネを作る実験を行なっている。Rakow (1972) の得た比較的低リノレイン酸の mutant をさらに EMS で処理し、Röbbelen と Nitsch (1975) は、リノレイン酸含量がより低く、リノール酸含量が高まった mutant を得た。

疑 応 答 質

谷野：講演要旨の 3 図の分類は系統とあまり関係がないようだがどういうことか。次にブランカの仲間はインドールの代謝においてきわめて変った行動をとる。ブランカというものは代謝から系統を見るという意味ではあまり使えないのではないか。第 3 にリノレイン酸の α 型、γ 型の分類はそれに系統を定めるのに有効なように思われる。(Erwin & Block) これを無視した理由を聞きたい。

角田：一番最後の α 型、β 型は文献に出ているが、今回は検討の対象とななかった。第 1 点については Thies がやった型分類と種子植物の系統樹との関係は明らかでないと言った方がよいと思う。しかし Thies は子葉種子と胚乳種子とで種子油の脂肪酸組成がちがうと見ている。子葉種子は種子の発育の少なくとも途中の段階あるいは成熟の直前までグリーンで光合成する。そういう子葉種子は種子油に不飽和脂肪酸を多く含んでいるように見える。具体例では、アマ、ナタネ、特に Thies はナタネについてそういうことを言っている。つまり胚乳体の構成要因として脂肪酸は重要であるが、その場合に膜とての透過性を高めるために相当不飽和でなければまずいという点と、光合成をしていると酸化的な過程が起こりやすいから種子に集積される脂肪酸の不飽和がますますという。二つのことを推定している。何かの形で光合成あるいは胚乳体と不飽和脂肪酸の形成に係るのではないかという。1968 年に型分類をした時には、このこととは言っていたのが最近になって別の突然変異系統を使った比較研究の一つとしてこのことを言っている。

私は推定ではあるが低温発芽性とか低温初期生育には不飽和脂肪酸をもっている種子の方がいいのではないか、低温の時不飽和の高い方がエネルギーとして使い易いから、そういう面で若千の適応があるのではないか。不飽和を進める遺伝子は主として遺伝子を通じて使われる。遺伝子は基本遺伝子によってもある突然変異により変り、これが耐えている限り何か生態適応という意味があるのではないか、第 2 の質問の意味がよくわからないが脂肪酸代謝でもブランカの仲間は、長鎖の脂肪酸形成をする点で、他の植物とかもっている。

高木：ナタネ、ベニバナで脂肪酸組成を大きく変える遺伝子が発見されているが植物の系統分化から考えて他の作物でこのような作用力の大きい遺伝子の発見は考えられない。
か。

角田：ナタネで成功しているのはエルシン酸関係だけである。ナタネ、大豆で大きな問題であるリノレイン酸含量を熱心にやっているが、スエーデンのスカロフのグループが相当の系統を調査して若干少ないものを見つけて交配育種をやっている。雑のものは見つけられていない。私がもがかったアブラナ科植物 53 種ではいままで観察されていない、種を相当含めてやっているが雑のものは見出されていない。そういうことで、もしそれが子葉種子の宿命的なものであれば少なくとも雑にすることはあきらめた方がよい。このあたり Thies と Röbbelen では若干意見を異にしている。ともかく、生理的代謝に脂肪酸組成がどういう具合に関与しているかを研究しなければならない。ごくまれに形成されるものを確実に取り出すために多く早く検定する技術は Thies あたり開発しているが、見通しとしてはナタネでは雑にするのは難しいが相当低めることは出来るのではないかと思われる。ダイズでも同じであろう。
HORMONAL ASPECT OF DWARFISM

Susumu Kuraishi

Department of Biology, College of General Education, University* of Tokyo, Komaba, Meguro, Tokyo 153, Japan

Introduction

The external application of gibberellic acid results in a faster rate of growth of pea (Brian and Hemming 1955, Lockhart 1956, Gorter 1961, Kende and Lang 1964), corn (Phinney 1956), Lurium (Cooper 1958), Cucurbita (Denna 1963) and watermelon (Loy and Liu 1974). However, the gibberellin treatment fails to overcome all the stunted growth to the normal one. Gibberellic acid markedly stimulates the growth of the seedlings of tall varieties, but not the growth of dwarf varieties of wheat (Allan et al. 1959, Radley 1970, Harada and Vergara 1972, Gale and Marshall 1973), and certain variety of corn (Phinney 1956) and barley (Hradilik 1973). Furthermore, seedlings of certain dwarf variety of corn (Phinney 1961), rice (Suge and Murakami 1968), pea (Kende and Lang 1964, Jones and Lang 1968) and wheat (Radley 1970) contain more or similar amount of gibberellin-like substances than those of tall variety. Thus, the gibberellin content in the dwarf mutants does not always reflect on the cause of dwarfism, although the importance of gibberellin in dwarfism should not be underestimated.

McComb and McComb (1970) reciprocally grafted the tall and dwarf variety of pea which responds to gibberellin and concluded that the slow growth variety is not dependent on endogenous gibberellin in the seedlings. Ogawa (1962 and 1965) found less amount of gibberellin in "Kidachi," a dwarf variety of a Japanese morning glory, than its contrasting normal type, although Barendes and Lang (1972) found no difference in gibberellin content between Kidachi and normal variety. Thus, present experiments were performed to see whether or not gibberellin is the cause of the dwarfism of Kidachi of a Japanese morning glory, using a technique of reciprocal grafts.

Gibberellin does not extensively stimulate the growth of coleoptiles (Ricard and Nitsch 1958, Hradilik 1973) and cannot be regarded as the major cause of coleoptilar dwarf. Van Overbeek in maize (1938) and Lantican and Muir (1969) in

* Present address: Department of Environmental studies, Faculty of Integrated Arts & Sciences, Hiroshima University, Hiroshima 730, Japan.
pea concluded that the cause of dwarfism is an abnormal auxin metabolism.

The pathway of auxin biogenesis has not yet established. Tryptophan as the main precursor of the IAA synthesis has been disputed, because of the low rate of tryptophan conversion to IAA in aseptic tissues and the non-specific enzymes involved (Winter 1966, Thimann and Grochowska 1968). Winter (1966) and Ishigami and Suzuki (1970) have shown that IAA was synthesized directly from indole and ethanolamine. Gibson et al. (1972a, b) showed a high rate of tryptophan conversion to IAA when cold tryptophan was supplied to the reaction mixture along with radioactive tryptophan. Erdmann and Schiewer (1971) presented an evidence that tryptophan is the native IAA precursor, since IAA synthesized in plant tissue with serine-\(^3\)H and indole-\(^14\)C is similarly synthesized from tryptophan which was labeled with serine-\(^3\)H and indole-\(^14\)C. However, this experiment seems to use tryptophan with low specific activity, since tryptophan labeled with serine and indole was extracted from tissue and added to the IAA synthesizing tissue. Transaminase which has a \(K_m\) value at the order of 10\(^{-3}\) M (Gamborg and Wetter 1963, Treulson 1972) forms indolepyruvic acid, which is spontaneously converted into IAA, when excess tryptophan was added. Thus, the double labeling experiment of tryptophan might be an artifact due to the application of high concentration of tryptophan.

Thus, present study was performed to find the possibility of pathway of the auxin biogenesis under the physiological concentration of tryptophan using a semi-brachytic barley variety.

A part of the present studies was reported in the earlier publication (Kuraishi 1974).

Materials and Methods

1) Plant materials

Seeds of dwarf variety of *Pharbitis nil* cv. Kidachi and its contrasting normal type, Tokyo-kokei, were supplied by Dr. T. Endo of the National Institute of Genetics, Mishima, Japan. The seeds were dipped for 30 min in concentrated sulphuric acid and washed thoroughly with tap water for 2 hr. The washed seeds were kept at 27 °C in the dark humidity room for 48 hr. Then, the seedlings were transplanted to small clay pots and raised in a green house (temperature above 15 °C) and watered daily.

A barley cultivar, Akashinriki, of the nzu or semi-brachytic type, and its contrasting normal type, isogenic except for the *nzu* gene, were supplied by Prof. R. Takahashi of the Ohhara Institute for Agricultural Biology, Okayama University.
The recessive gene \((uz)\) exerts diminishing action of the coleoptile and many other areas, including the stem (Takahashi 1942, 1947).

Seeds of *Avena sativa* cv. Victory 1 were obtained from Svalov, Sweden. Seeds of both *Hordeum* and *Avena* were imbibed at 27 °C for 24 hr under weak red light to prevent growth of mesocotyls (Larsen 1955), then, they were transplanted into sand and kept at 27 °C for another 48 hr in the total darkness. For the study of the auxin extraction of barley coleoptiles, seeds were sown in wet vermiculite for 80 hr at 27 °C in the dark.

2) **Grafting techniques**

The first epicotyls next to the hypocotyls of a Japanese morning glory were selected as the region of grafts. Half epicotyl segments, *ca.* 7 mm in length, were cut off. Then two plants to be grafted were tightly connected with their fresh cut surfaces by 3 ties of cheese cloth. Within one week after the operation, one of the scions and stocks were cut at the grafts.

3) **Sterile culture**

Seeds were imbibed in 10% chlorinated lime for 5 min under an aspirator vacuum, then they were soaked in the same solution under normal pressure for another 25 min. Approximately 100 of these seeds were washed 6 times with

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![Graph](Fig. 1. Growth of *UZUZ* and *usuz* coleoptiles grown in sand. Seedlings were grown at 27°C in the dark. Contents are the average of 10 coleoptiles. (Kuraishi 1974)]
sterilized water and placed in 100 ml of sterilized 0.75% solidified agar in a 500 ml Erlenmeyer flask. Seeds were kept at 27 °C for 24 hr under weak red light, then transferred to total darkness for another 48 hr at 27 °C. In both sterile and non-sterile cultures, at the end of the growth period, the coleoptile length of UZUZ and uzuz was ca. 3.0 and 1.5 cm, respectively (Fig. 1).

4) **Extraction of auxin**

About 70 g of coleoptiles with the first leaves were excised, homogenized with 300 ml cold methanol, and extracted with 300 ml of methanol twice at 4 °C for 48 hr. Extracts were combined and evaporated to a small volume under reduced pressure. This concentrate was fractionated into a neutral and an acidic fraction according to Larsen's modification (Larsen 1955) of the Boysen-Jensen method (Boysen-Jensen 1941). The neutral and acidic fractions were obtained by successively shaking the concentrate with peroxide free ether at pH 8.0 and 3.5.

5) **Paper chromatography**

The acidic fraction was divided into two equal aliquots. An aliquot of the acidic fraction was concentrated and streaked on Toyo No. 51 filter paper, 2 cm wide, from the edge of the paper and was developed by ascending chromatography in isopropanol: ammonia (1.1 M): water (10:1:1 v/v/v) for 15 hr at room temperature. Authentic IAA was added to another aliquot of the extract to provide a comparison of the Rf of the extractable auxin with the Rf of IAA. After drying the filter paper, the portion of the chromatogram between the origin and the solvent front was cut into 10 equal portions and each portion was placed in a glass vial. Each piece of cut chromatogram was eluted with 1.0 ml of water for 30 min at 60 °C. Then 12 agar blocks (2×2×2 mm) were placed in the water extract and equilibrated for 3 hr. The auxin content in the agar blocks was assayed using the standard *Avena* curvature test.

6) **Bound auxin**

The residue of the methanol extract was dried, then hydrolyzed in 25 ml of 1 N NaOH at 100 °C for 1 hr (Klämbt 1961). This hydrolysate was assayed for auxin as described above.

7) **Basispetal transport**

The basipetal transport of IAA was determined by the method of van der Weij (1932). Carboxyl labeled IAA, 0.5 mCi/mM, Radiochemical Centre, Amersham, England, was dissolved in 1 ml of 2×10⁻⁴ M IAA aq. solution, making a final spec. act. of ca. 2.5 mCi/mM. The diluted radioactive IAA solution was solidified with agar (1.5%, final concentration) and cut into 2×2×2 mm blocks. These donor blocks were placed on the apical cut surface of a 5 mm coleoptile section cut 2 mm below the tip, after removing the first leaf. Receiver blocks of 1.5% agar (2×2×2
mm) were placed on the basal surface. After an appropriate transport period, the receiver blocks, the donor blocks, and the coleoptile segments were rapidly frozen in liquid nitrogen, then crushed by a glass rod. The radioactivity in each portion was measured with a Packard tri-carb liquid scintillation counter, using Bray's solution. The counting efficiency was about 80%.

8) Conversion of tryptophan and tryptamine to IAA

Aseptically grown harley seedlings, 3.0 cm and 1.5 cm in the coleoptile length of UZUZ and uzuz, respectively, were used. Fifty coleoptile tips, 5 mm in length were floated on 2 ml solution containing 10 μCi of radioactive precursors of IAA and kept at 27 °C in the dark. After the incubation period of 3 hr, the coleoptile tips were washed with tap water and homogenised using a glass homogenizer. The extractants used were 80% ammonium sulphate according to the method of Atsumi et al. (1976). The extract was adjusted to pH 3.5 with tartaric acid and extracted thrice with dichloromethane. The dichloromethane fraction was washed with water (pH 8.0: NaHCO₃). The water fraction was acidified to pH 3.5 and extracted with dichloromethane thrice. The dichloromethane fraction was applied to Whatmann 3 mm paper and developed in the same solvent system of the extraction of auxin. The paper was cut into equal 25 pieces and radioactivity was measured in a Packard tri-carb liquid scintillation counter, using Bray’s solution. 9)

9) Tryptophan

Tryptophan was extracted according to the method of Morris (1955) and its content was estimated colorimetrically (Spies and Chambers 1948, 1949).

Results

1) Grafting experiments

Reciprocal grafts were made between Kidachi and Tokyo-kokei, when their length was 6.5 cm and 21.0 cm, respectively. A solution of 10⁻⁴ M gibberellin

<table>
<thead>
<tr>
<th>Stock</th>
<th>Scion</th>
<th>Scion length (cm)</th>
<th>H₂O-treatment</th>
<th>Gibberellin-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tall</td>
<td>Tall</td>
<td>13.6±3.0</td>
<td>15.5±3.5</td>
<td></td>
</tr>
<tr>
<td>Dwarf</td>
<td>Tall</td>
<td>15.5±3.5</td>
<td>32.3±7.3</td>
<td></td>
</tr>
<tr>
<td>Dwarf</td>
<td>Dwarf</td>
<td>3.5±1.3</td>
<td>24.3±6.5</td>
<td></td>
</tr>
<tr>
<td>Tall</td>
<td>Dwarf</td>
<td>3.9±1.0</td>
<td>22.3±7.2</td>
<td></td>
</tr>
</tbody>
</table>

Plants were grafted at the time of 2 weeks old and length of scion was measured 3 weeks after grafts. A concentration of 10⁻⁴ M gibberelic acid or water was poured around the roots 2 weeks after the grafts.
poured around the roots of stocks brought about a marked increase in rate of shoot growth, demonstrating passage of gibberellin across the graft union. Growth of the scion was not affected by the stock, indicating no interaction of scion growth with stocks, either presence or absence of gibberellin treatment (Table 1). Gibberellin has shown to be produced in roots and transported through vascular system (Philips and Jones 1964, Jones and Philips 1966 a and b, Skene 1967, Atsmon et al. 1968). Movement of gibberellic acid applied externally to the growing region suggests that the gibberellin produced at the roots and cotyledons has no effect on the growth of scion. However, present experiment cannot rule out the possibility of the intervention of gibberellin produced in the apices. Thus, the next experiment was undertaken to see the effect of the apecies on the elongation of lateral bud using reciprocal grafted Japanese morning glory.

Solution of 1% kinetin (Sachs and Thimann 1964) was applied to the cotyledonary node (stock) after 2 weeks of grafts. Cytokinin-induced elongation of lateral bud was measured one week after gibberellin treatment (2 weeks after cytokinin-treatment) (Table 2). Almost no significant elongation of cytokinin-induced

<table>
<thead>
<tr>
<th>Stock</th>
<th>Scion</th>
<th>Length of lateral buds (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H₂O-treatment</td>
</tr>
<tr>
<td>Tall</td>
<td>Tall</td>
<td>0.6 ± 0.3</td>
</tr>
<tr>
<td>Dwarf</td>
<td>Tall</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Dwarf</td>
<td>Dwarf</td>
<td>0.7 ± 0.3</td>
</tr>
<tr>
<td>Tall</td>
<td>Dwarf</td>
<td>0.6 ± 0.3</td>
</tr>
</tbody>
</table>

Solution of 1% kinetin was applied 2 weeks later of grafts and 10⁻⁴ M gibberellic acid or water was treated after the cytokinin-treatment. Length of lateral bud was finally measured one week after the last treatment.

lateral bud elongation was seen regardless of the difference in the scion and stock. Treatment of gibberellin to the plants stimulated the elongation of lateral bud. Enhancement effect on the lateral bud of dwarf and tall variety were of the same magnitude. Here again, scion did not significantly alter the growth of lateral bud. The fact that scion has no effect on the elongation of lateral bud suggests that gibberellin content in the variety of Japanese morning-glory does not act as a major cause of their dwarfism.

2) Growth of barley coleoptile

The growth of UZUZ and uzuz coleoptiles grown in sand is shown in Fig. 1. Growth of the UZUZ coleoptile was greater than that of the uzuz coleoptile even on the first day after imbibition. On the 4th day after imbibition, the first leaves in
both strains protruded from the coleoptiles, thus, the growth of coleoptiles was slowed down. The growth curve data for coleoptiles grown on vermiculite or sterilized agar at 27 °C in the dark was identical to the data shown in Fig. 1.

3) **Auxin content in the coleoptiles**

In preliminary experiments, the apex of the coleoptile was excised and placed on a 2×2×2 mm agar block in the dark for 3 hr to collect diffusible auxin. No appreciable diffusible auxin could be detected from the tips either the UZUZ or usuz coleoptiles, when assayed using the *Avena* curvature test. Thus, 5 coleoptile tips were placed on an agar block of the same size and kept for 3 hr at 27 °C in the dark. Still no appreciable curvature was observed in the agar block.

Seventy grams of 3 day old whole UZUZ and usuz coleoptiles, 3,000 and 5,000 segments, respectively, were harvested and extracted with methanol. No auxin activity was observed in the neutral fraction from either the UZUZ or usuz coleoptiles.

The auxin from the acidic fraction of the extract of UZUZ coleoptiles seems to be IAA, since the Rf of the acidic auxin corresponded to the Rf of authentic IAA. The *Avena* curvature assay showed that the amounts of auxin in the UZUZ and usuz coleoptiles were respectively, ca. 1.3×10⁻⁵ and less than 5×10⁻⁷ IAA equivalent (g)/fresh weight (kg) (Table 3). The usuz coleoptile contains far less auxin than the UZUZ coleoptile.

<table>
<thead>
<tr>
<th></th>
<th>Free auxin</th>
<th>Bound auxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>UZUZ coleoptiles</td>
<td>1.3×10⁻⁴</td>
<td>5.5×10⁻⁶</td>
</tr>
<tr>
<td>usuz coleoptiles</td>
<td>5.0×10⁻⁷</td>
<td>1.8×10⁻⁶</td>
</tr>
</tbody>
</table>

Each figure is significant at the 0.01 level. (Kuraishi 1974)

The bound auxin was extracted with ether from the methanol insoluble fraction after alkaline hydrolysis and was assayed using the *Avena* curvature test. The amount of bound auxin from the UZUZ and usuz coleoptiles were 5.5×10⁻⁶ and 1.8×10⁻⁶ IAA equivalent (g)/fresh weight (kg), respectively (Table 3). The data indicate that UZUZ coleoptiles contained approximately 3.4 times more bound auxin than did usuz coleoptiles. These experiments suggest that the usuz coleoptiles contains far less total IAA than the UZUZ coleoptile.

4) **Transport and destruction of exogenous auxin**

The stunted growth of the usuz coleoptile may be due to a decreased ability to transport auxin from the tip to the growing region or by high activity of IAA oxidase.
The velocity of auxin transport was measured by the method of van der Weij (1932). The decrease in the auxin level during auxin transport was assayed by counting the decrease in total IAA-\textsuperscript{14}C in the receptor and donor blocks, and in coleoptile cylinders. Since preliminary experiments using barley coleoptiles showed only a very small amount of transport, transport in \textit{Avena} coleoptile segments was examined for comparison with the transport in barley coleoptiles.

After removing the first leaf from inside the coleoptile cylinder, an agar block containing IAA-\textsuperscript{14}C was placed on the apical surface. Changes in the radioactivity of the coleoptile cylinders, donor blocks, and receptor blocks were measured over a 6 hr period (Fig. 2). Radioactivity due to IAA-C\textsuperscript{14} in coleoptile cylinders of both \textit{UZUZ} and \textit{uzuz} was maximum at ca. 3 hr, but continued to increase over the 6 hr

**Fig. 2.** Decrease in IAA-\textsuperscript{14}C during transport in a coleoptile cylinder, 0.5 cm long. Contents are average of 10 plants. A: Radioactivity in the coleoptile cylinder. B: Decrease in radioactivity in donor blocks. C: Changes in radioactivity in receptor blocks. D: Total radioactivity (Kuraishi 1974)
period in *Avena* (Fig. 2A). The ratios of the decrease in radioactivity in donor blocks in both *Avena* and barley coleoptiles were of the same magnitude (Fig. 2B). This suggests that the 3 kinds of coleoptiles absorb auxin from the donor blocks at approximately the same rate. The amount of radioactivity which diffused into the receptor block of the barley coleoptile was far smaller than that into the *Avena* coleoptile (Fig. 2C), therefore, the barley coleoptile may have higher oxidase activity than the *Avena* coleoptile. The sum of the radioactivity in the receptor blocks, donor blocks, and coleoptile cylinders are shown in Fig. 2D. The decrease in total radioactivity represents the amount of auxin destruction during transport. This decrease in radioactivity was least in the *Avena* coleoptile. When the decrease in radioactivity during transport was measured on the basis of coleoptile length, the *usz* coleoptile showed the highest activity for auxin destruction. However, if the decrease was expressed as the radioactivity/unit of fresh or dry weight, the decrease in radioactivity in both barley coleoptiles was of the same magnitude; the *UZUZ* coleoptile had about 30% more fresh and dry weight/unit length than did the *usz* coleoptile. Thus, the difference in the amount of auxin between the *UZUZ* and *usz* coleoptiles cannot be expressed by the difference in IAA oxidase activity in both tissues.

5) **Auxin biosynthesis**

The precursor of auxin is transported from the endosperm and is converted into the active principle at the apex of the coleoptile (Skoog 1937). The exogenous

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**Fig. 2.** Time course of the growth of coleoptiles due to IAA and its precursors. Seedlings were grown under sterile conditions and floated on sterilized medium. 0.5 cm Coleoptiles with apices intact were floated on the medium. IAA: $10^{-3}$ M. Tryptamine and tryptophan: $10^{-3}$ M (Kuraishi 1974)
applied precursor of auxin will stimulate the growth of isolated coleoptile apex. Thus, the coleoptile with apex was fed with either auxin or auxin precursors, such as tryptophan and tryptamine. The UZUZ coleoptile apex responded to IAA, tryptophan (only at 10⁻⁸ M concentration) and tryptamine (Fig. 3), but the uzuz coleoptile apex responded only to tryptamine and not to tryptophan. This suggests that the UZUZ coleoptile can convert tryptophan to IAA more readily than the uzuz coleoptile, only when high concentration of tryptophan was furnished.

Radioactive tryptophan at 10⁻⁶ M concentration was fed to both UZUZ and uzuz coleoptile apices. Chromatographic evidences showed no conversion to IAA⁻¹⁴C from tryptophan⁻¹⁴C of low concentration in both apices so far as 3 hr incubation period, although most of the radioactivity due to tryptophan was incorporated into protein (data was not furnished). On the other hand, tryptamine⁻¹⁴C at same low concentration as tryptophan⁻¹⁴C fed to coleoptile apices of UZUZ and uzuz was equally incorporated into IAA⁻¹⁴C region (Fig. 4.) The result suggests that there is no difference of the conversion of tryptamine to IAA between two kinds of coleoptile apices.

![Graph showing radioactivity due to tryptamine-¹⁴C](image)

Fig. 4. Paper chromatography of radioactivity due to tryptamine⁻¹⁴C. Radioactivity tryptamine was fed to UZUZ and uzuz coleoptiles with apices at 27°C for 3 hr in the dark. Acidic CH₃Cl₂ fraction of the extracts was developed with isopropanol: ammonia: water

6) **Tryptophan content in barley coleoptiles**

Tryptophan content was measured after collecting 500 coleoptile cylinders, and shown in Table 4. Both UZUZ and uzuz coleoptiles contained similar amount of
tryptophan at the order of $10^{-5}$ M. Since the endogenous level of tryptophan in both coleoptiles is quite low, transaminase to form indolepyruvic acid may hardly acts in these tissues because of the $K_M$ value of transaminase.

**Discussion**

A number of dwarf mutants are known to induce a normal type growth after the treatment with gibberellin and also contain less gibberellins in the plant tissue. However, some dwarf mutants such as pea and Japanese morning glory, which responded to gibberellin cannot be easily explained their cause of dwarfism by the low level of gibberellin in the tissue. McComb and McComb (1970) used reciprocal grafts and found on change in shoot phenotype as a result of grafts. Present results also showed no interaction between scion and stock using a Japanese morning glory, when gibberellin is applied to the root system. Furthermore, cytokinin-induced elongation of lateral buds is stimulated by the externally applied gibberellin, but again, the growth of lateral buds are not affected by the fast-growing scion, regardless the external application of gibberellin. If the cause of fast-growing scion of *Pharbitis* is a high level of gibberellin in the tissue, the endogenous gibberellins are assumed to be transported to the lateral buds at the cotyledonary nodes and results in the growth stimulation of the lateral buds. Thus, present results cannot support the cause of dwarfism of Kidachi as a results of the high level of gibberellins, although the external and endogenous gibberellins might have a different effect on the cytokinin-induced elongation of lateral buds. Gibberellin content in the plants does not always reflect the elongation, since non-transportable gibberellins in the cells may not have much effect on the elongation of the intact plants, until they are converted into transportable forms. Thus, the experimental technique of the reciprocal grafts with special references to the cytokinin-induced bud elongation would be useful for the elucidation of the cause of dwarfism.

Gibberellin does not extensively stimulate the growth of coleoptile (Ricard and Nitsch 1958, Hradilík 1973) and cannot be regarded as the main cause of the coleoptilar dwarf. The present study also revealed that semi-brachytic barley, *uzuz*, has less amount of auxin than its contrasting normal type. Thus, the importance in the auxin economy for the investigation of the cause of coleoptilar dwarf should
be emphasized. Apical tips of the normal coleoptile grown under the sterile conditions responded to both tryptophan and tryptamine, but \textit{mut} coleoptile tips responded only to tryptamine. However, both coleoptile species hardly converted tryptophan-\textsuperscript{14}C to IAA-\textsuperscript{14}C, but easily converted tryptamine-\textsuperscript{14}C to IAA-\textsuperscript{14}C. Although 10\textsuperscript{-3} M tryptophan is necessary to induce the elongation of the normal coleoptile, tryptophan level in the tissue was only the order of 10\textsuperscript{-5} M. A Km value of transaminase which convert tryptophan to indolepyruvic acid was ca. 10\textsuperscript{-3} M (Gamborg and Wetter 1963, Trelinson 1972). Thus, endogenous tryptophan at 10\textsuperscript{-5} M would not hardly be converted into indolepyruvic acid, which is spontaneously degraded into IAA (Gordon 1961). A pathway of decarboxylation of tryptophan to IAA is another candidate for the biosynthesis of auxin, although the distribution of decarboxylase in the plant kingdoms is limited (Gibson \textit{et al.}, 1972 b). Thus it would be necessary to investigate the formation of IAA from other precursors, since the externally applied tryptophan has no effect on the aseptically grown \textit{Avena} coleoptiles (Thimann and Grochowska 1969, Winter 1966). The use of dwarf mutant which produce less auxin would bring a new field for the elucidation of the pathway of auxin biosynthesis.

Acknowledgement

The author wishes to express his thanks to Mrs Rumiko Yoshino, Miss Kayoko Kurosawa and Mr. Takashi Iwasaki for their technical assistances.

Summary

Reciprocal grafts between dwarf (Kidachi) and normal type (Tokyo-kokei) of a Japanese morning glory revealed that the growth of scion was not affected with the stock in the presence or absence of externally applied gibberellin. Cytokinin-induced elongation of lateral bud at the cotyledonary nodes of the reciprocal grafts was stimulated by the applied gibberellin. However, the difference in the stock or scion did not affect the elongation of lateral buds, regardless of the application of gibberellin. These results suggest that the cause of the stunted growth of Kidachi is not simply less amount of gibberellin in the tissue.

Coleoptile of a semi-brachytic barley, \textit{mut}, contained less amount of auxin than those of \textit{UZUZ}. This less amount of auxin in the \textit{mut} coleoptile was not due to changes in the rate of basipetal transport of auxin, neither to the destruction of auxin during transport, not sensitivity to auxin, suggesting that the less amount of auxin is due to less production of auxin. Normal coleoptile responded both
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trypotphan and tryptopamine, but szsz responded only to tryptamine. However, both UZUZ and szsz coleoptiles did not convert tryptophan to IAA when physiological concentration of tryptophan was furnished. This suggests the presence of the pathway of auxin biosynthesis which does not pass through a transamination in barley coleoptiles.

References


矮性形態出現と植物ホルモン

倉 石 見*

東京大学教養学部生物学教室

植物体内のジペレリン含量不足が矮性形態の出現理由として述べられているが、ジペレリン含量だけが矮性形態を支配するものではない。アサガオの矮性品種“木立”とジペレリンの関係については異論が多く、まだ確立されていない。木立と東京古型（正常）を交互つぎ木して、台木はつぎ芽の生長に影響を及ぼさず、またジペレリン処理はつぎ芽の生長を促すが、台木による差は現れなかった。台木の子葉節にカイネチン処理を行なった、腋芽の伸長を促進させることが可能であるが、台木やつぎ芽の差による腋芽のジペレリンによる生長差は認められなかった。このことはアサガオの木立は単なるジペレリン含量が少ないために矮性化したとは結論がたい。

ウズ遺伝子をもつ半矮性オオムギ子葉鞘はオーキシン含量が少なかったが、これはオーキシンの破壊や移動の差によって生ずるものではなく、オーキシンの生産量が少ないためと思われる。オーキシンの前駆物質であるトリプトファンやトリプタミン溶液に子葉鞘の先端を浮かべると正常なる葉鞘では高濃度（10⁻³ M）のトリプトファンとトリプタミンで生長の促進が見られたが、ウズ遺伝子を持つ葉鞘ではトリプトファによる生長促進作用は見られなかった。ウズ遺伝子の存在にかかわらず、放射性トリプタミンは放射性インドール酢酸を生成したが、両子葉鞘とも、放射性トリプトファンからのインドール酢酸の生成は見られなかった。子葉鞘中のトリプトファン含量は10⁻⁶ M 程度であることがから、トランスアミナーゼは通常の条件下では作用しないと考えられる。これらの結果からインドール酢酸生成の生合経路はトリプトファン以外の前駆物質を考慮する必要がある。

質 疑 応 答

中島：リンゴでは樹冠部を矮性化させる台木がイギリスのエストモーリン研究所で実用化しているが、サクラなどその他の樹木についても地上部を出来るだけ矮性化させるような突然変異体を誘発させてほしいという話題が数年前、永年生作物の放射線育種に対するIAEA の会議で出されたが、こういう突然変異体を選抜していく過程では植物生理学的にどういう点に留意したらいか。

例えばジペレリンレベルや、IAA で選ぶのかあるいは細胞学的にいて蛋白質で選ぶのか。

* 現在：広島大学総合科学部
倉石：今までの矮性のもののうち有望なもののがどのようなタイプであるか調べてみた上でないと難しい。

松尾：中島氏に聞きたいが、サクランボの矮性（自然突然変異かもしれない）についてジベレリンなどを調べたものはいるか。

中島：サクランボのCompact Lambertは人為突然変異品種として登録されているが、それに関する調査はない。オイルペア、ゴムの木などを矮化させた方がいいという勧告があるがどうしたらいか方法がない。

松尾：日本の果樹で矮性台木を使っているものがあるのではないか、そのような台木はどのような性質か。

池田：両高を低くするというやり方の中に台木の方を小さくする、いわゆる根系の方を小さくする方法と、地上部を小さくしていく方法、接木部のコンピネーションによる方法の三つの場合が考えられ、矮化台木のホルモン産成とともにアブサイシン酸の高産成で説明されている論文が多い。しかし接木部のコンピネーションによる方法では接木部の転流などが問題であって、今のところホルモン関係だけでは解釈出来ないのではないかと思う。

池田：キクチアサガオの接木実験でジベレリンとジベレリンの反応系という形でジベレリンを測る方がよいということだが、外から与えた場合と生体がもっているジベレリン濃度との関係の試験をすすめる場合にどのような注意をしたらいか。

倉石：全部やってみる必要がある。まずぶっかけてみてその変化をみること、その上で含量を調べ、接木実験をするという順序でやっていくのが最終的な結論を出すよい道だと思う。私の観察したものの中に気孔が開いたままで、しおれたうが原品種よりはるかに生育がいいものがあった。特にグリーンハウス環境下で生育させる時には気孔を開かせるということが有効だと思うので、もしこのような突然変異があった場合には持らさずに子どもの所へ持って来てほしい。これは最近のグリーンハウスのもとでの試みとして興味がある。
UTILIZATION OF ARTIFICIAL MUTANTS FOR FUNDAMENTAL RESEARCHES ON HERBICIDES

Shooichi Matsunaka*

Division of Plant Physiology, Department of Plant Physiology and Genetics, National Institute of Agricultural Sciences, Konosu, Saitama 365, Japan

Introduction

To the study of biochemistry, especially to the elucidation of metabolic pathways in organisms, artificial mutants have made great contributions.

On the other hand, chemical weeding has completely changed the style of weeding in the modernized agriculture, and the clarification of the mode of action or selectivity mechanism of herbicides is getting to be more important for the purpose of both effective and safe use of them.

Fortunately thousands of mutants have been produced from the Norin No. 8 rice variety in the 3rd Laboratory of our institute, and the author was allowed to use these mutants by courtesy of the laboratory. Then the author tried some experiments to use the artificial mutants of rice plants in the fundamental researches of herbicidal mechanisms.

Utilization of chlorophyll mutants

1) Chlorophyll mutants

Among the mutants obtained from Norin No. 8, there are CM-marked ones (chlorophyll mutants) which are defective in the biosynthesis of chlorophyll (including defect in formation of chloroplasts), and the color of them ranges from pure white to yellow and to light green. The physiological characteristics of these chlorophyll mutants were reported in another paper (Saka and Matsunaka, 1975).

2) Light-requiring mechanism of diphenylether herbicides

Herbicides of the diphenylether group are in wide use, being applied to more than 2.5 million ha (in total of CNP, chlomethoxynil, and nitrofen) of paddy fields

* Present address: Department of Plant Protection, Faculty of Agriculture, Kobe University, Rokkodaicho, Nada-ku, Kobe, Hyogo 657, Japan.
in 1974. Among this group of herbicides, the ones which have substituent radical at the ortho-position are inactive in the dark; that is, the light is necessary for their herbicidal action. Other diphenylether herbicides which have no substituent groups at the ortho-positions (as like as HE-314 or DMNP) are active in the dark and have another mode of action.

In the first place, reactions of the above-mentioned white and yellow mutants which had no chlorophyll with nitrofen were examined. When just germinated seeds were placed in Petri dishes with the test herbicide and put under a fluorescent lamp, it was observed that yellow seedlings were influenced by nitrofen, while pale yellow or white ones were not affected by the chemical even in the light condition. The growth of green segregant (segregated in the ratio of white-yellow 1: green 3) was completely inhibited by the treatment. Of course, viridis (light green) seedlings also are affected by the herbicide. An example of the results is shown in Figure 1 (Matsunaka, 1969).

Then pigments were extracted from each kind of mutants and analyzed by thin layer chromatography. CM 213, which was a mutant susceptible to nitrofen in the light condition, contained no chlorophyll but xanthophyll at nearly normal
concentration. White mutants did not contain any pigment, and light yellow ones had no chlorophyll but about 10% of the normal concentration of xanthophyll. These results show that xanthophyll is at least one of the light-receptors in the light-requiring mechanism of nitrofen.

Other diphenylether herbicides having ortho-position substituent (CNP, chlome-thoxynil and others) showed the same pattern as nitrofen. Oxadiazone (17623-RP, G-315, 2-tert-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-5-oxo-1,3,4-oxadiazoline) is a herbicide quite different in chemical structure from the diphenylether group, but it has a characteristic action similar to nitrofen and others, and the reaction of the chlorophyll mutants to this chemical is the same as to nitrofen (Matsunaka, 1970).

3) **Mode of action of bipyridylum herbicides**

The use of bipyridylum herbicides, especially paraquat and diquat, increases every year because of their strong and quick effect by the contact treatment of
leaves and stems as well as the absence of residual effect in the soil.

The mechanism of their action has relation to the photosynthetic system as shown in Fig. 2., that is, electrons are excited in photochemical systems I and II when the photosynthetic organs absorb the light, and the excited electron acts on paraquat molecules to reduce them to change into free radicals. The paraquat free radicals are oxidized by oxygen in the air and the original form of the molecules is recovered. At the same time an excessive power in the oxidation of free radicals produces activated oxygens which will kill the leaves and stems of treated plants (Asada, 1978).

Therefore, the efficacy of paraquat is remarkably reduced in the dark or oxygen-free condition or when it is coexistent with the Hill-reaction inhibitors even in the light condition.

When paraquat is applied to the above-mentioned chlorophyll mutants, green seedlings wilt within the day of treatment and turn brown on the next day, while yellow or white ones begin to wilt about three days after the treatment. Herbicidal activity of paraquat was very weak on the yellow seedlings of CM 213 and others unlike nitrofen, indicating that not only light-receptors but also the photosynthetic systems is necessary for the photoactivation of paraquat.

**Propanil-susceptible rice mutant**

1) **Selection and characteristic features of the propanil-susceptible mutant**

Rice plants are highly resistant to the herbicidal action of propanil (3',4'-dichloropropianilide). Therefore, propanil can be applied directly on rice plants to control barnyardgrass (*Echinochloa crus-galli*) and other weeds growing in rice fields such as direct sowing rice culture. It is believed that this selectivity is due
to the presence of an enzyme which catalyze the hydrolysis of propanil in the rice plants. The propanil-hydrolyzing enzyme, a kind of aryl acylamidase (Frear and Still, 1968), has been of interest since this enzyme is found in rice plants but not in barnyardgrass. However, rice plants differ considerably from barnyardgrass even in their morphological characters. Therefore, it seemed somewhat unreasonable to attribute the resistance of rice plants to propanil entirely on the presence of the hydrolyzing enzyme in rice. To confirm this point, the author examined about 50 cultivated rice varieties, including foreign varieties, on their tolerance toward propanil. None of the 50 varieties was susceptible as barnyardgrass to propanil.

The author attempted to find a propanil-susceptible rice plant among the artificial mutants. He was allowed to spray about 700 different lines of rice mutants with propanil in exchange for weeding their nursery beds by courtesy of the 3rd Laboratory of Genetics. Just prior to spraying, the mutant rice plants were morphologically examined. One week after spraying, careful examination of the plants showed that seedlings of the mutant No. 408 and No. 409 were almost entirely killed by the spraying of propanil. The remained seeds of No. 408 were given to the author for subsequent propagation. The mutant No. 408 was created by 32P inner-radiation of seeds from Norin No. 8.

Strain No. 408 was differentiated from the parental variety as a mutant because of its decreased number of tillers as compared to the parent. The growth of the mutants strain was poor under normal conditions of fertilization. The time of heading was 5 days later in the mutant than in the parent. The leaves of the mutant was also narrower and tended to curl slightly in hot weather. Needless to say the most characteristic feature of the mutant was its propanil-susceptibility. The mutant rice was equally susceptible to propanil as barnyardgrass in the 3 to 4 leaf stage of growth. The parent, Norin No. 8, was totally resistant to propanil at the same growth stage.

Normal rice plants are able to hydrolyze propanil as shown in Fig. 3. A large
amount of 3,4-dichloroaniline (DCA) is produced when propanil is incubated with a homogenate from rice leaves or stems for about one hour.

The author assayed the activity of the propanil-hydrolyzing enzyme, aryl acylamidase, in the mutant by measuring the production of 3,4-dichloroaniline as well as the disappearance of propanil itself. The results indicated that the propanilsusceptible rice mutant had almost no propanil-hydrolyzing activity. In addition to propanil itself, $2',3'$-, $2',4'$-, $2',5'$-, $2',6'$- and $3',5'$-dichloropropionanilides also could not be hydrolyzed by homogenates of the mutant. Therefore, it may be concluded that the aryl acylamidase is not present in the mutant. Recently, Akatsuka and Kasakura (1972) reported that the mutant contained an aryl acylamidase with a different specificity. Its best substrate was $2',3'$-dichloroacetanilide and not propanil.

Photosynthesis in normal rice plants was completely inhibited within two hours after spraying with propanil. However, a slow recovery occurred, and about one week later the photosynthetic activity nearly equal to that of the control. The photosynthetic activity in the propanil susceptible mutant did not recover after a similar treatment (Matsunaka, 1971). It is reasonable to conclude that propanil was hydrolyzed and detoxified by normal rice plants over the one week period. In contrast, the absence of photosynthetic recovery in the propanil-susceptible mutant reflects the inability of mutant rice to hydrolyze and detoxify propanil. These results strongly suggest that the mutant rice plants are susceptible to propanil because of the absence of the hydrolyzing enzyme, aryl acylamidase, in the plant body.

2) Genetic background of the mutant

Crossing experiment were performed to elucidate the genetic behavior of the mutant character. By crossing the resistant rice R (Norin No. 8), to the susceptible rice, S (mutant), a considerable number of $F_2$-seeds, $R \times S$ and $S \times R$ were obtained. All hybrids obtained from the $R \times S$ and $S \times R$ crossing were far more resistant to

<table>
<thead>
<tr>
<th>Table 1. $\chi^2$ analysis of the segregation ratio of the $F_2$-plants</th>
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<tbody>
<tr>
<td>After application of propanil number of plants</td>
</tr>
<tr>
<td>Survival</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Observed (O)</td>
</tr>
<tr>
<td>Calculated (C)</td>
</tr>
<tr>
<td>$O - C$</td>
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</tbody>
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$\chi^2 = \frac{(O - C)^2}{C} = 0.0454$

$P = 0.90 - 0.80$

$304 : 899 = 1 : 2.96$
propanil than S, the susceptible mutant.

\( F_1 \)-seeds collected were planted in a growth cabinet to obtain \( F_2 \)-seeds. From a total of 1,203 \( F_2 \)-seedlings from both initial crosses of \( R \times S \) and \( S \times R \), the plants segregated in a ratio of 1:2.96 (susceptible to tolerant). These results were analyzed by \( \chi^2 \) test (Table 1) which showed an agreement with the theoretical ratio of 1:3. These results suggested that the susceptibility to propanil was a genetic character controlled by a single recessive gene (Matsunaka, 1974).

\( F_2 \)-plants were tested individually for aryl acylamidase activity. Out of 83 \( F_2 \)-plants tested, 21 plants showed no aryl acylamidase activity. This ratio of segregation (21:62) appeared to be consistent with the theoretical ratio of 1:3.

\( F_2 \)-plants were further numbered individually and a single leaf from each plant was removed and assayed for the enzyme activity. The plants were then sprayed with propanil to determine if only the enzyme-negative plants would be killed by the propanil treatment. The results showed that only the aryl acylamidase-negative plants were susceptible to propanil injury. The relationship between propanil-susceptibility and the absence of enzyme was more directly proven by this experiment.

The \( F_1 \)-individuals mentioned above were grown and used for backcrossing with the susceptible mutant, S. The offspring thus obtained were resistant and susceptible in the ratio of nearly 1:1, giving further positive proof of the monogenic inheritance of propanil-susceptibility (Table 2).

<table>
<thead>
<tr>
<th>Propanil hydrolyzing enzyme (aryl acylamidase)</th>
<th>Presence</th>
<th>Absence</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed (O)</td>
<td>24</td>
<td>32</td>
<td>56</td>
</tr>
<tr>
<td>Calculated (C)</td>
<td>28</td>
<td>28</td>
<td>56</td>
</tr>
</tbody>
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\[
\chi^2 = \frac{(O-C)^2}{C} = 1.14 \\
P = 0.30 - 0.20
\]

**Table 2. \( \chi^2 \) analysis of the segregation ratio of the backcross, \( F_1 \times S \)**

**Conclusion**

Besides the above-mentioned experiments, Shirakawa (1970) investigated the herbicidal mechanism of solan (3'-chloro-2'-methyl-\( \beta \)-valeroluidide) and found that white or yellow mutant rice is resistant to the herbicide. He assumed on the basis of this result that the action of solan had some relation to the photosynthetic
system.

The chlorophyll mutant rice appears to be useful not only for the study of herbicides but also for the elucidation of light-dependent physiological and biochemical reactions in general.

On the other hand, the propanil-susceptible mutant rice, which was discovered very fortunately, gives us useful information about the selective action of propanil, and if we come to think of the fact that the propanil-hydrolyzing enzyme is an enzyme which has been present in normal rice since the time when the herbicide was not yet known, we expect that the enzyme has some physiological significance. This point may also be clarified by a comparison between Norin No. 8 and the mutant. The results will be useful informations in the fields of plant nutrition.

Acknowledgement

The author wishes to express his deep thanks to Dr. T. Kawai for his kind helps during this study.

References

除草剤の作用機構研究における突然変異系統の利用

松 中 昭一*
農業技術研究所生産遺伝部
埼玉県鶴ヶ島市鶴ケ島 1, 227

生化学の研究、とくに物質代谢経路の解明の研究に、微生物等の突然変異株の利用が大きく貢献したことは既に実証された。ひろがって、最近の除草剤の普及はめざましいものがあり、たとえば、水田作では全作付面積に毎シーズン2回以上施用されていると計算される。したがって、その効率的利用および安全性確保のために、除草剤の作用機構や選択性機構を明確にしておく必要があり、著者は、わが国で使用されている主要な除草剤についてその検討を行っている。ここでは当研究所遺伝科において創製されたイネ突然変異系統を利用したこの分野の研究結果を報告する。

CNP やクロスキシニール、あるいは NIP のグループ、ジフェニルエーテル系除草剤は、わが国で最も多量に使用されているものであるが、いずれも殺草性を発揮するために光を必要とする。そこで、この場合の光の受容体箇所であるかを検討するために、水稲染米 8号の葉緑素突然変異系統のうちキサンチン（黄色）やアルビネ（白色）にたいする除草剤 NIP (nitrofen) の挙動を調べた。その結果、白色のものは、この除草剤と光とが共存してもその作用をうけないが、黄色のものは緑色のものと同様に大きな被害をうけることが明らかになった。黄色の突然変異系統は、かなりの量のキサンチンを含み、これらジフェニルエーテル系除草剤の作用機構における光受容体のすくなくともその一つはキサンチンであると結論された。

除草剤オキサジアゾンは、その化学構造がジフェニルエーテル系のものと著しく異っていても、光を要求する点や低毒性、あるいは葉鞘発芽変生など生理作用がきわめて類似している。この除草剤についても、葉緑素突然変異株を利用して検討した結果、白色株はその作用をうけないのに黄色株は緑色のものと同様に殺草作用をうけることが判明し、この点でも上記ジフェニルエーテル系除草剤と同様の作用機構を示すことが明らかとなった。

一方、パラコートやシクロロートなどの、ビリジリウム系除草剤も殺草にあたって光要求性（暗所では効力が激減する）である。通常の緑色植物はパラコート散布後光があれば数時間以内に枯死しほじめる。しかし、前述の黄色株や白色株では枯死までに3日程度を必要とする。このことは、パラコートの殺草作用が、光合成の光化学系 I, II により生ずるラクト型バイオリンの還元（フリーラジカル化）とその空気中の酸素による酸化にともなって生ずる活性酸化物がもとづくという説を強く支持するものである。

もうひとつの突然変異株は除草剤 DCPA（国際的には propanil）に関するものであ
る。この除草剤にたいしてイネは特異的に強い選択性を示すため、直接栽培のようなイネと雑草が同じ条件で共存する場合でも無差別散布でイネに害を与えることなく雑草を枯殺することができる。この選択性機構として、イネがもっている DCPA 分解酵素（一種の aryl acylamidase）による解毒が考えられている。

著者は、当研究所遺伝第3研究室で水稲農林8号から育成された突然変異系統約700について DCPA 受験性を検討した結果、2系統がイヌナビエなどとほぼ同程度の受験性を示すことを見出した。このうち1系統を増殖し、種々の検討を行なった。普通のイネであれば、DCPA 散布後直ちに阻害される光合成も徐々に回復し、すくなくとも1週間後には完全に無処理区程度まで回復する。ここで得られた受験性系統は、DCPA 散布後、光合成が阻害され続け、回復はみられない。一方、この受験性系統は、普通のイネがもつ上記 DCPA 分解酵素を含まないことも判明した。

そこで、親系統農林8号と本受験性系統との交配実験をした結果、F1 は DCPA 抵抗性、F2 で抵抗性：感受性＝3：1 の分離を示し、F1×感受性の重交種でもほぼ 1：1 に分離し、DCPA 感受性にかんして、単因子劣性の遺伝をすることが判明した。また、F3 において、各個体における上記 DCPA 分解酵素の有無と DCPA 散布後の生存・枯死とは完全に一致することがわかった。以上のこととは、普通のイネがもっている DCPA への抵抗性は、DCPA 分解酵素の有無に支配され、この酵素の生成能は単一遺伝子に依頼していることを示している。

以上のように、突然変異株は、単に除草剤作用機構解明のみでなく、光生物学的現象（葉緑系突然変異系統）や窒素代謝系（DCPA 感受性系統）の解明などにも活用できるよう。

質疑応答

相賀：ジェフェニール・EHコールと光の働きについて青い光が一番効果的で、赤い光が少し効いて緑の光がほとんど効かないという話をあったが赤い光がある程度効くということとキサンツフィールの関与を教えてほしい。つまり青い光の効果の中の紫外線の働きはどうだろうか。キサンツフィールが直接にレセプターとなるかどうか。

松中：緑の光がほとんど効かず、赤い光が少し効くのはやはり葉緑素の吸ったエネルギーが移動するために考えられる。その辺の光のエネルギーの役割が葉緑素の中にあるのではないかと思う。また紫外線そのものではないとやっていないが、色つきの蛍光灯を用いた前向きフィルターなどでうまくみた結果、可視部のいわゆる青い光が効いているようである。

相賀：アクションスペクトルの数が何か特定のキサンツフィールの吸収スペクトルと一致するということはどうか。

松中：特定のキサンツフィールにあり程度をかみかみ厳密にはやっていないのだからわからない。

相賀：赤い光が効くのはクロロフィルが何か関係していると考えてよいのではないか。

松中：そう考えてよいと思う。
武田：DCPA 感受性の 408 と 409 は姉妹系統ですか。

松中：我々もそのような感じをもらったが種子を入手したのは 408 だけだった。しかもあらためて聞いた時関係がないと聞いたが、その辺はっきりしない。409 の種子をもらって相互関係を調べてみたい。

武田：この春フジニオリのエチレンイミンの処理後代にも DCPA 感受性系統が出てい るので、もし 408 と 409 が別のソースだとすると易変的な遺伝子と考えるか。

松中：先ほど倉石氏は応用面での発展を話されたがそれは抵抗性を失う方向だから応用面 では問題にならない。あえていえば解毒の機構の中で雑草の方は A なら A という化合 物を活性化する性質、例えば MCPB の CH₃COOH を切りはずし側鎖を酢酸にする系す なわち、β-oxidation の能力の強いものは自分で毒物を作って枯れてしまう。このような 能力の弱いものは活性化がでないので助かるというものです。そういう例が見つかれば そういう活性化機構をもっている雑草は枯れる。したがってある作物だけそういう機能を なくしてしまうと助かるということになり、突然変異である酵素系を欠損させることでか えって抵抗性になって役に立つというのもあろうかと思う。このような例は非常に少なく て先の表のように解毒、分解あるいは結合を起こす系の方が毒物の解毒系としては多いよ うなので応用面での利用価値は少ないかと思う。

渡辺：藤巻氏が遺伝的雄性不稔をやっているが細胞質雄性不稔は 100% 雄性不稔になる からかいが、遺伝的雄性不稔の場合は 3:1 で劣性なので 25% しか維持出来ない。この 場合感受性のものを正常の方に豊富させておけば、苗代の時これを散布することによって 雄性不稔だけを残すことができるという意味で価値があると思う。

角田：DCPA を解毒する酵素をイネのみが持つと本文に書いてあるが、厳密に Oryza sativa だけが持つものか、Oryza 属のものがもつということで禾本科の他のものは持っ ていないか。

松中：グラスペラでやってみたがこれは持っていた。その他特殊なものにはそれぞれあ る。例えばチューリップの球根などはかなり持っている。ところがちょっと性格が異って イネの酵素はいわゆるパウンドタイプでミクロゾーム的な小さな顆粒にくついているが 他の植物でみつかっているのは可溶性の型でかなり性格がちがうので DCPA 解毒に相当 に役立っているのはイネのこの酵素だけである。いわゆるアリアルシルアミダーゼ 全般を考えるとむしろ別の性格のものの方が植物全般には分布しているようだ。今まかい 点は筑紫大学の赤塚先生がイネの酵素だけでなくアリアルシルアミダーゼ全般にわたって 解説されているのでそれを参考にしてはしない。（植物酵素・蛋白質研究法、蛋白質・核 酸・酵素・別冊、p.160、1976)
TEMPERATURE SENSITIVE CHARACTERISTICS
IN THE PHENOTYPIC MANIFESTATION
OF RICE CHLOROPLAST MUTANTS

Ichiro Aiga,* Takeshi Omura** and Hikari Sato**

* National Institute for Environmental Studies, Ibaraki, Japan
** Faculty of Agriculture, Kyushu University, Fukuoka, Japan

Introduction

Various kinds of mutants having defects in chlorophyll biosynthesis or chloroplast morphogenesis have been noted in many plant species.1) Some of them reveal stable phenotypes under various environmental conditions, and the phenotype of others change from white or yellow leaf colour to normal green colour by alteration of environmental factors such as temperature, lighting condition and nutrients.2–5) In rice plants, many chlorophyll deficient mutants have been described; however, studies on the interaction between genotype and environment are few.6) Hundreds of chlorophyll deficient mutants of rice have been preserved in the Plant Breeding Laboratory, Faculty of Agriculture, Kyushu University. Recently, the CM number mutants which were obtained by the treatment of chemical mutagenic agents, were added to the collection.7)

The phenotype of some mutants grown under paddy field condition often depends on the sowing time, climate and other factors; consequently, there is considerable confusion in identification of the mutant. The use of advanced environmental control facilities (Phytotron) which provide precise control of temperature, moisture, lighting and other environmental factors, has enabled us to analyze the influence of environmental factors on the phenotypic manifestation.

In this paper, several strains of temperature sensitive chloroplast mutants in rice were classified according to a simple system based on leaf colour. Using v1, v2 and v3 strains, the influences of alternating temperatures and nitrogen nutrients on the phenotypic manifestation are described.

Materials and Methods

Materials: Rice, *Oryza sativa*, chloroplast mutant strains in the collection were used. CM number strains were induced from a paddy rice variety "Kimmaze"
by treatment of \( n \)-nitroso-\( n \)-methylurea.\textsuperscript{8}) F1 191 is a hybrid progeny of an American rice variety "Arkarose virescens" introduced by Dr. Jodon of U.S.D.A. HO799 is a spontaneous mutant from a paddy rice variety "Yaeho." Seeds of HO799 were generously provided by Prof. Takahashi, Okayama University. The respective genes for the virecent of F1 191 and HO 799 have been identified as \( v_1 \) and \( v_2 \) and they belong to the linkage group XI.\textsuperscript{9,10} The gene for the virecent of CM13, designated as \( v_0 \), belongs to the linkage group I.\textsuperscript{11}

The phenotype of \( v_1 \) seedlings grown under field conditions varied remarkably with sowing time. When \( v_1 \) seedlings were grown in early April in the Kyushu Area, the third leaves were completely white and it was impossible to distinguish \( v_1 \) seedling from albino seedlings. They were nonviable. The white part of the third leaves gradually decreased as the sowing time became later, and the leaves finally became green and were viable when the seeds were sown in early July. Temperature sensitive mutants are hard to distinguish from each other because they show similar phenotype in lower temperature season.

Culture conditions: All experiments were carried out in artificial light growth cabinets. The seedlings were grown at given temperatures with a precision of \( \pm 0.5 \)°C under continuous irradiation using a metal halide lamp (Toshiba Yoko-Lamp) or a daylight fluorescent tubes. The illuminance was about 6,000 lux (Yoko-lamp 400 W) or 2,500 lux (Fl. tubes 40 W×2). The relative humidity was kept at 65 \( \pm \)5%.

Unless otherwise stated, the culture medium used was a modified White's medium containing \( \text{MgSO}_4\cdot\text{7H}_2\text{O} \) 739.5 mg, \( \text{Ca(NO}_3\text{)}_2\cdot\text{4H}_2\text{O} \) 287.8 mg, \( \text{Na}_2\text{SO}_4\cdot\text{10H}_2\text{O} \) 458.8 mg, \( \text{KNO}_3 \) 80.0 mg, \( \text{KCl} \) 65.0 mg, \( \text{Na}_3\text{PO}_4\cdot\text{2H}_2\text{O} \) 21.4 mg, \( \text{MnSO}_4\cdot\text{6H}_2\text{O} \) 7.18 mg, \( \text{ZnSO}_4\cdot\text{7H}_2\text{O} \) 2.16 mg, \( \text{H}_3\text{BO}_3 \) 1.5 mg, \( \text{KI} \) 0.75 mg, Fe-Citrate 15 mg, powdered agar 6 mg and distilled water 1,000 ml. Glass pots containing the nutrients and agar were autoclaved at 120 °C for 15 minutes.

Hulled seeds were surface sterilized by immersing them successively in 80% ethanol for 3 minutes, in 10% chlorinated lime for 20 minutes, in 3% hydrogen peroxide for 20 minutes and then rinsed several times in sterile water. The seeds were germinated at 25 °C for 48 hours and grown in growth cabinets.

Chlorophyll determination: The amounts of chlorophyll \( a \) and \( b \) in the third leaves were used as an index of the phenotype. Three to five leaves of the mutant seedlings were harvested. The leaves were ground in a mortar with 80% aqueous acetone and the extract was centrifuged. The supernatant and re-extract from the residue was mixed. A known volume of combined supernatant was used for the colorimetric determination of chlorophylls \( a \) and \( b \), using the specific absorption coefficient of Mackinney.\textsuperscript{12}
Results and Discussion

Classification of temperature sensitive chloroplast mutants

Some temperature sensitive strains in the chloroplast mutant collection were grown in a growth cabinet at successive constant temperatures of 20 and 30 °C, at a relative humidity of 65% under continuous illumination of about 6,000 lux. The seedlings were cultured for 30 days at 20 °C and 18 days at 30 °C after germination. The leaf colours of the seedlings showed in Table I. The mutants were classified

Table 1. Temperature sensitive rice chloroplast mutants

<table>
<thead>
<tr>
<th>No.</th>
<th>Strains</th>
<th>Leaf colour grown at 20°C</th>
<th>Leaf colour grown at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>1</td>
<td>FI 175, FI 191, CM 73, HO 799, CM 278, CM 282</td>
<td>W Albina</td>
<td>G</td>
</tr>
<tr>
<td>2</td>
<td>CM 13, CM 247</td>
<td>W Albina</td>
<td>PY</td>
</tr>
<tr>
<td>3</td>
<td>CM 155, CM 170, CM 247</td>
<td>W Albina</td>
<td>PG</td>
</tr>
<tr>
<td>4</td>
<td>CM 156</td>
<td>PG Viridis</td>
<td>W</td>
</tr>
<tr>
<td>5</td>
<td>CM 214</td>
<td>G Normal</td>
<td>W/G</td>
</tr>
<tr>
<td>6</td>
<td>CM 14</td>
<td>Y Xantha</td>
<td>G</td>
</tr>
</tbody>
</table>

*1 leaf colour in 2nd to 5th leaf.
*2 according to Gustafsson et al. (1962)
W; White, PG; Pale green, G; Green, Y; Yellow, PY; Pale yellow

according to the changes in leaf colour at each temperature. The leaf colour of group 1 become completely white at 20 °C, while at 30 °C, normal green colour appeared in the leaves of the same strain seedlings. Consequently, seedlings of identical strains were distinguishable by being albino at low temperature, and normal green at high temperature.

Gustafsson et al.\(^1\) proposed classification of chloroplast mutants in Phanerogams for practical purpose. They classified them according to a simple system based on leaf colours. In their classification of colour changing plants, the effects of environmental factors on the phenotypic manifestation must be considered.

Temperature sensitive characteristics of \(v_1\), \(v_2\) and \(v_3\) seedlings

The amounts of chlorophylls in expanded leaves of \(v_1\), \(v_2\) and \(v_3\) seedlings grown at 20, 25, 30 and 35 °C under continuous illumination of 2500 lux are shown in Fig. 1. Larger amounts of chlorophylls were observed at the higher temperatures in all three mutant strains, but the temperature effects differed somewhat among
the three mutants. In \( v_1 \) seedlings, there was only a trace of chlorophylls at 20 °C, while, at 25 °C, the chlorophylls reached 2.2 mg/g.f.w., nearly the same as at 30 °C, and the leaf colour was mostly green. In \( v_2 \) seedlings, the chlorophylls and leaf colour at 20 °C were about the same as those of \( v_1 \) seedlings grown at 20 °C. At 25 °C, the amount was only 70% of that at 30 °C, and the tip of third leaf remained partly white. At 30 °C, the chlorophylls reached 2.3 mg and green colour appeared in the leaf. In \( v_3 \) seedlings, the amount of chlorophylls was very small even at 25 and 30 °C. Pale yellowish white leaves were observed at 30 °C. At 35 °C, the leaf colour became pale green and the chlorophylls reached 1.6 mg.

The ratio of chlorophyll a to chlorophyll b was about 3:1 regardless of the total amount in all three mutant strains. The absorption spectra in the visible light region in 80% aqueous acetone extract showed the same pattern in each case described. The evidence suggests that the change in the chlorophylls is accompanied by a similar change in the carotenoids. For this reason, the amounts of the chlorophylls were used as an index of the phenotype in the mutants.

As \( v_1 \) seedlings grown at 20 °C differed greatly from those grown at 25 °C with respect to chlorophyll content, the influence of temperature was examined in detail. As shown in Fig. 2, the amount was negligible below 22 °C, but began to increase rapidly at 22 °C and reached a plateau at 25 °C in \( v_1 \) seedlings. Therefore, it was determined that the threshold temperature preventing chlorophyll formation was 22 °C in \( v_1 \) seedlings. Similarly, threshold temperature for \( v_2 \) and \( v_3 \) were found to be 20 °C and slightly below 30 °C, respectively.
Fig. 2. Amount of chlorophylls in the third leaves of \( v_1 \) seedlings grown at various temperatures

Temperature sensitive mutants have been reported in maize by Miller and McWilliam\(^2,3\) and in barley by Miller and Zalik.\(^4\) Low temperature sensitivity seems to be a common characteristic of three mutant plant species, barley, maize and rice. Miller and McWilliam\(^2\) showed that the maize mutant had a threshold temperature for chlorophyll accumulation, approximately 17 °C, and at this temperature, although the chlorophylls was essentially zero, the development and morphology of the plant was normal and seedlings die only when seed reserves were exhausted. A rapid increase of chlorophylls in the narrow temperature range from 22 °C to 25 °C seems to be characteristic of rice mutants, especially \( v_1 \) seedlings.

**Modification of temperature sensitivity by nitrogen nutrients**

In the experiments hitherto mentioned, modified White's medium was used. In the course of the experiments, if medium containing only agar was used in place of White's medium, the greening of the seedlings was stimulated. Table II shows

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>( v_1 )</th>
<th>( v_2 )</th>
<th>( v_3 )</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W A</td>
<td>W A</td>
<td>W A</td>
<td>W A</td>
</tr>
<tr>
<td>20</td>
<td>0.04 1.03</td>
<td>0.23 1.50</td>
<td>0.00 0.00</td>
<td>3.18 2.78</td>
</tr>
<tr>
<td>25</td>
<td>2.12 3.34</td>
<td>1.78 2.80</td>
<td>0.06 0.04</td>
<td>3.50 2.94</td>
</tr>
<tr>
<td>30</td>
<td>2.44 3.22</td>
<td>2.46 3.11</td>
<td>0.44 1.27</td>
<td>3.33 3.13</td>
</tr>
</tbody>
</table>
the amount of chlorophylls in \( v_1 \), \( v_2 \) and \( v_3 \) seedlings grown on medium containing only 0.6% agar and Fe-Citrate (A-Medium) and on White's medium (W-Medium) at various temperatures. All three strains had more chlorophylls in the A-Medium than in the W-Medium at every temperature, though the normal strain had slightly more in the W-Medium than in the A-Medium. It also was found that medium containing only NO\(_3\)-compound showed similar effects to the W-Medium.

The effects of NO\(_3\)-compound on the amount of chlorophylls in \( v_1 \) seedlings grown at 18, 20, 24 and 26 °C were examined by the application of KNO\(_3\) in concentration of 0 mg/l (N-0), 75 mg/l (N-75), 300 mg/l (N-300) and 1200 mg/l (N-1200). The NO\(_3\) concentration in N-300 was approximately that of the W-Medium. The results are shown in Fig. 3. The NO\(_3\) effect varied with temperature, showing no effect at 18 °C and the highest effect at 22 °C. This shows that the chlorophylls decrease as the NO\(_3\) concentration in the medium increases. As a result of the NO\(_3\) effect, the threshold temperature mentioned above, was shifted to a higher temperature as the NO\(_3\) concentration in the medium increased. It was found, moreover, that NH\(_4\) compound, urea and glycine reacted similarly.
In general, nitrogen compounds are well known for their role in the acceleration of chlorophyll accumulation, but in the rice mutants, it seems that chlorophyll accumulation is partially inhibited by nitrogen nutrients. Nitrogen nutrients affected the expression of genes by shifting the threshold temperature upward proportional to the amount. The shift of the threshold temperature by KNO₃ was exerted only in a particular temperature range. From the evidence, it is probable that nitrogen nutrients do not play a major role in gene expression, but only modify the effect of temperature.

**Specific temperature sensitive growing stage on the phenotypic expression of genes**

As mentioned above, the phenotype of each mutant was remarkably influenced by temperature conditions. If the temperature of the culture was altered from 20 °C to 30 °C or vice versa after the leaves were fully expanded, the phenotype remained unchanged by this temperature treatment. However, alternating the temperature during the initial leaf growing period distinctly influenced the phenotypic manifestation as follows: Each day for nine days after germination, lots of $v_1$ and $v_2$ seedlings precultured at 20 °C, were transferred to a growth cabinet maintained at 30 °C. Successively, these seedlings were grown until the third leaves of the seedlings were expanded. The expanded leaves were harvested and the chlorophylls content

![Graph showing the relationship between periods of preculture and chlorophyll content.](image)

**Fig. 4.** Amount of chlorophylls in the third leaves of $v_1$ and $v_2$ and length of the third leaves of $v_1$ after preculturing at 20°C.

- ○○○○, chlorophylls in $v_1$.
- ○○○, chlorophylls in $v_2$.
- ----, length of third leaf of $v_1$. 2E and 3E, stages at second and third leaf emergence.
was determined. As shown in Fig. 4, the chlorophylls of the \( v_1 \) seedlings precultured at 20 °C for one day was 2.57 mg, nearly the same amount as for those grown at 30 °C from the initial germination period. The chlorophylls decreased rapidly as the duration of the preculture period became longer at 20 °C, reaching about 0.5 mg when the seedling were precultured at 20 °C for five days. The growth stage of the seedlings at one day and five days after germination corresponded to second and third leaf emergence, respectively. The third leaf was 1.6 mm and 14.4 mm long, respectively. The results of the opposite case, transferring \( v_1 \) seedling from 30 °C to 20 °C, are shown in Fig. 5. The amounts of the chlorophylls were, contrary to the results of Fig. 4, very low when the seedlings were transferred after the third leaf emergence. Chlorophylls in the third leaves at the stage of third leaf emergence were undetectable at 20 °C and even at 30 °C.

From the above evidence, it may be speculated that a specific temperature sensitive stage for the determination of the phenotypic manifestation exists in an initial short period of the leaf emergence. It is interesting that the ability to form chloroplast in the developed leaf is determined during a short period of the leaf emergence. It seems that \( v_1 \), \( v_2 \) and \( v_3 \) mutant strains might be blocked genetically.
in some unknown initial stage in the sequence of chloroplast morphogenesis. The unknown stage seems to be temperature sensitive.

As a practical application, the temperature sensitive characteristics for the phenotypic manifestation may be utilized as biological index for delicate changes in the temperature of the environment surrounding a paddy field.

Data concerning to $v_1$, $v_2$ and $v_3$ cited from the report by Omura et al. (J. Fac. Agr. Kyushu Univ. 21. 1976. in press).

**Summary**

1. Seedlings of temperature sensitive rice chloroplast mutants were grown at constant temperatures of 20 and 30 °C successively. Mutant strains were classified according to the phenotypic manifestation that corresponded to each temperature.

2. Temperature sensitive characteristics of $v_1$, $v_2$ and $v_3$ seedlings were examined. The threshold temperature preventing chlorophyll formation was found to be 22 °C for $v_1$, 20 °C for $v_2$ and slightly below 30 °C for $v_3$.

3. The amounts of chlorophylls in the seedlings decreased as nitrogen nutrient in the medium increased. The threshold temperature became higher as the application of nitrogen nutrient increased.

4. A specific temperature sensitive stage during the growing period was found. In the third leaves of $v_1$ seedlings, only a short period from the second leaf emergence to the third leaf emergence was temperature sensitive. No chlorophylls were detected in the third leaves at the stage of third leaf emergence.

5. From the above evidence, it seems that $v_1$, $v_2$ and $v_3$ mutant strains might be blocked genetically in some unknown initial stage in the sequence of chloroplast morphogenesis. The unknown stage seems to be temperature sensitive.

**References**


5. **Walles, B. (1963):** Macromolecular physiology of plastid IV. On amino acid requirements of


7. **Omura, T.:** Unpublished data

8. **Omura, T.:** Unpublished data


11. **Omura, T.:** Unpublished data

相賀一郎，大村 武，佐藤 光

質 疑 応 答

河合：葉緑素含量と温度の関係曲線での立上がり点は突然変異体によって異なるか。また温度の関係で葉緑素形成のクリティカルステージは業によって決っているか。

相賀：$v_1$については20℃、$v_2$では25℃、$v_3$では30℃がクリティカルである。また第2点のクリティカルステージは葉ごとにあるようだ。
MUTANTS OF RICE, SOYBEAN AND VEGETABLES

Sachihiko Tanaka, Nobuhiko Nagata
and Susumu Hiraiwa

Institute of Radiation Breeding, N. I. A. S.

Most radiation induced mutants with drastic changes of morphological and/or physiological characters tend to have morformed plant types and it is hardly expected that these ones contribute directly toward the development of a new variety in each crop. However, it is well known that some of them can play an important role as materials for physiological studies. In this point of view, the authors present some peculiar mutants of rice, soybean and vegetables in this paper, as these ones were shown in slides at the 15th Gamma Field Symposium.

In rice, nearly one thousand mutants of Norin 8 and six hundred mutants of

Fig. 1. Mutants with changed panicle of rice
a; gigantic, b; long, c; awned, cont; original variety
“Norin 8”, d; dwarf with shrinked grains, e; dwarf with compact grains.
Fig. 2. Hairless mutants seeds (upper) and normal seeds (lower) of rice var. "Nihonmasari."

Fig. 3. Color mutants of soybean: a; original variety "Tachisuzunari" (brown hilum), b; yellow hilum, c; brown seed-coat, d; brown saddle, e; partial brown saddle, f; white saddle.

Nihonmasari have been induced by physical or chemical mutagens. Norin 8 is an old variety produced by duplication of chromosome complement of a haploid plant and Nihonmasari is a new leading variety in Kanto district. Frequency of chlorophyll mutations of rice is rather high with the ratio of $4 \times 10^{-6}$/R and proportion of chlorophyll mutants to all the visible mutants is approximately forty per cent. Some of them could be used as materials for physiological studies, because heterogeneous plants are easily propagated vegetatively and most of their mutated
characters are controlled by a single recessive major gene. Mutants with necrotic spots are also frequently induced and most of them are viable. Among them, we have found a peculiar mutant of which leaves are active for a week during its maturing period, but endosperm of seeds is produced with the aid of chlorophyll in lemma until harvesting period, and its germination rate is over eighty per cent. Morphological mutants are usable as materials for physiological studies. For example, dwarf mutants have been determined for their gibberellin response by many workers. When artificially induced mutants with changes of morphological characters are used as materials for physiological studies, care should be taken, because, in many cases, they have a pleiotropic gene. Generally, artificially induced mutants such as dwarf, hairless, liguleless, neckleaf, rolled leaf, brittle culm, lazy and so on are very similar to each spontaneous mutants reported previously, however, it is expected to induce an unique gene mutation, because an induced dwarf mutant having very similar phenotype to a spontaneous one is controlled by a single gene which is different from a gene controlling the spontaneous one.

In soybean, morphological mutations were more frequently induced than physiological ones. Morphological mutants such as dwarf, short stem, seed colour, leaf shape and so on were induced by both chronic irradiation of growing plants and
acute irradiation of dry seeds. High protein mutants of Tachisuzunari were also induced by chronic irradiation of growing plants.

In tomato, mutants such as green hypocotyl, changed leaf shape, self-topping plant type, male sterile and so on have been isolated.

In sweet pepper, mutants with changes of leaf shape such as pine type, heart type and spinach type were rather frequently induced. A chlorophyll mutant has leaves which is yellow leaf colour at its young stage and gradually turn to green. Several male sterile mutants were induced by chronic irradiation of growing plants.
BRIEF DESCRIPTIONS ON MUTANTS IN
VEGETATIVELY PROPAGATED
AND TREE CROPS

Hisashi Kurimura, Fukio Ikeda, Haruhiko Fujita
and Takehiko Maeta

Institute of Radiation Breeding, N.I.A.S.

Within recent years, a plenty of mutant clones have been recovered along
with the course of studies of methodology in mutation breeding of vegetatively
propagated and tree crops at the Second Breeding Technique Laboratory in the
Institute of Radiation Breeding, N.I.A.S. From among these mutant clones of
fruit trees, mulberry, forest trees and tuber crops, some mutant clones which may
attract any interests of plant physiologists or botanists will be briefly described.

Fruit trees

IRB 500-2  Small fruit mutant of apple cv. Ralls: This clone originated from
chronic gamma-irradiation (11.4 kR, ca. 45 R/day) in 1964, and was characterized
by spur type growth and attained about one third of tree height compared with
that of the original.

IRB 500-14  Ever vegetative mutant of apple cv. Fuji: This clone originated

Fig. 1. IRB 561-2 Spine-off mutant of Sunki mandarin,
Right; the original, left; the mutant
from semi-acute gamma-irradiation (10 kR, 1 kR/day) in 1967, and was characterized
by dwarf type growth and having round tree shape. Grafting compatibility with
Maruba-Kaido (Malus prunifolia Borkhausen var. Kingo Asami) root stock is un-
favourable and grafting union exhibits overgrowth of the top.

IRB 561-2  Spine-off mutant of Sunki mandarin (Citrus sunki Hort. ex Tanaka):
This clone originated from nucellar seedlings by acute gamma-irradiation (4 kR for
20 hrs) in 1974 and characterized by weak tree growth, yellowish leaf colour and
short internode (Fig. 1). Spine-off character in Citrus seedlings is very rare in general.

Mulberry

IRB 240-11  Garyū shoot mutant:  This clone was induced by acute gamma-
irradiation (10 kR, 250 R/hrs) to the dormant bud of an induced mutant IRB
240-4 (leaves 5-lobed) in 1972. The original clone IRB 240-4 was induced from
cv. Ichinose which was 5-lobed leaves predominantly, green, glossy and smooth on
the upper surface, straight shoot and semi-vigorous. Garyū shoot is characterized
by its zig-zag shape.  Fig. 2 shows one of the four buds developed after cutting-back
treatment.

IRB 240-12  Petiole twine mutant:  This clone was induced by chronic gamma-
irradiation (13 kR, 8.46 R/day, from 1969 to 1975), developed from adventitious
bud on a stem by cutting-back treatment.

Forest trees

IRB 601-4  Pendulous with long needle mutant of Kuma-sugi (local variety of
Cryptomeria japonica D. DON):  This clone was induced by acute gamma-irradiation
(600R). Although treatment with GA₃ on Sugi is usually effective on its flower induction, this clone does not respond to the treatment at all.

**IRB 601-6** Dwarf with thin and short needle mutant of Kuma-sugi: This clone was induced by acute gamma-irradiation (600R). It exhibits non apical dominancy and treatment with GA₃ is ineffective on flower induction.

**IRB 601-13** Dwarf with tricussate phylotaxy mutant of Kuma-sugi: This clone was induced by acute gamma-irradiation (600R).

**IRB 601-20** White flush type mutant of Kuma-sugi: This clone was induced by acute gamma-irradiation (400R). White flush in needle leaves appears at early stage of growing season.

**IRB 601-22** Dwarf waxless mutant of Kuma-sugi: This clone derived from a spontaneous sport from waxless mutant IRB 601-21 which was induced by chronic gamma-irradiation. This clone exhibits exceptionally intense apical dominancy and tolerates to excess water in soil. Rooting ability of cuttings is rather low and flower induction by GA₃ treatment is feasible (Fig. 3).

![Fig. 3. IRB 601-22 Dwarf waxless mutant of Kuma-sugi](image)

**IRB 601-42** Waxless with thin and densely attached needle mutant of Iwao-sugi (local variety of *C. japonica*): This clone was induced by chronic gamma-irradiation (15.8 kR).

**IRB 601-65** Dwarf with thin and short needle mutant of Boka-sugi (local variety of *C. japonica*): This clone was induced by chronic gamma-irradiation (41.2 kR). Distinctive feature of this mutant is its juvenile form leaves.

**IRB 611-2** Juvenile form mutant of Hinoki (*Chamaecyparis obtusa* ENDL.):
This clone was induced by chronic gamma-irradiation (11.5 kR) from a seedling of Nagano clone of Hinoki. Leaves maintain ever juvenile form like as Himuro (C. pisifera ENDL. var. squarrosa BEISSN.) and do not dvelope to normal leaves of adult age.

IRB 611-4  Pendulous type mutant of Sawara (C. pisifera ENDL.): This clone was induced by chronic gamma-irradiation (11.2 kR). Apical dominancy is rather weak than the original and GA₃ treatment is ineffective on flower induction.

Sweet potato

IRB 122-5  Dwarf mutant induced by acute gamma-irradiation (15 kR, 375 R/hrs) on shoot of cv. Nōrin 1. Phenotypic expression of this clone resembles closely spontaneous mutant Tachi-Nōrin 1. Flowers are easily induced by grafting onto dwarf morning-glory (Kidachi Asagao).

IRB 122-16  Dwarf mutant induced by acute gamma-irradiation (30 kR, 500 R/hrs) on a seedling from a cross Kyū-kei 19-1001 times cv. Koganesengan. (Fig. 4).

![Fig. 4. IRB 122-16 Dwarf mutant of sweet potato](image)

IRB 122-17  Dwarf mutannt induced by acute gamma-irradiation (40 kR, 2 kR/hrs) on a seedling from a cross Kyūshū 58 times cv. Koganesengan.

Both the clones, IRB 122-16 and 122-17 have thick leaves and are very compact with extremely short internodes. Tuberization is rather poor. Density of stomata and size of guard cell are nearly equal to those of the originals. Chromosome aberration or aneuploidy might be responsible for the changes.
総合討論

座長 大曽根兼一
佐々木睦男

佐々木：只今から作物の遺伝・生理・生態研究における突然変異の利用について総合討論にうつりたい。生物学の研究方法は大ざっぱに物理化学的な方法と、突然変異体を使う比較的方法とに分けられると思う。メンドルはエンドウの突然変異体を利用することにより遺伝学の基礎を確立したが、遺伝学、生物学の発展の跡をたどってみると、生物学研究の主流は突然変異体をうまく利用するところにあるような気がする。生物の特性としての自己複製、形質発現あるいはそれの調節等についていろいろとわかってきたが、今後の生物（作物）の研究に突然変異体がどのように利用できるかについて討論をお願いしたい。

大曽根：まず座長をされた方々に分担されたところのポイントなりコメントなりを出し Jacquard ていただいているとつらかったしたい。

藤井：山口氏の講演の一部は突然変異の修復に関する問題で、突然変異の初期過程は DNA に対するいろいろの障害である。それは DNA のベースが変わるか切断があるとか、化学的、たとえば EMS のようにアルカリ化する等の型がある。生物は進化の過程で生命を保持するために障害の自己修復という能力を備えた。大きな意味の修復は植物の枝を切ると又別の枝が出てくるということもあるが、分子レベルの修復も当然ある。DNA に起こった損傷自体はそのまま突然変異的原因にもなるが、最近の知見ではこの損傷が修復する場合のエラーによっても突然変異が起こることが微生物レベルで明らかにされて来た。山口氏は高等生物において染色体レベルでの修復が、体細胞の染色体の組換えによって起こり、体細胞組換えをしないと思われるパーシャルアシナプスの突然変異頻度が高いということを示された。次に山形農試の渡辺氏との共同研究で興味があったのは対照とした突然変異型が細葉で、これについて同化量、病虫害抵抗性など色々な性質を分析され、多分 1 遺伝子の多面的発現であるとのことであったが、この問題は突然変異体について異なった角度から分析すれば、有用性質も見出される可能性を示したもので、突然変異利用を考える上で有益な示唆だと思う。角田氏は脂肪酸の合成と系統分化の関連を話された。これから感じたことはもとより合成系をもっていないものに対して、突然変異によって合成系をもたせようとするのは無理なことで、ある合成系ステップを高めるとかあるいは合成系を変更させるということに突然変異育種の可能性があるように感じた。

河合：倉石氏は矮性に色々なパターンがあるということを指摘され、ジェレリン (GA), インドール酢酸 (IAA), アブサインシン, 細胞壁など関与する四つの場合に分けて話された。GA については GA だけすべての矮性の問題を片付けられないことを例示された。浄性オオギの子葉柄の生長が IAA 量に依存していることを示されたが、特に IAA の
合成経路に関してトリプトファン系を確立されたことは、突然変異体を使った新しい知見として興味深い。アブサイシンについては、アブサイシン酸のないトマトの矮性突然変異体が紹介された。細胞壁の問題では、大麦の矮性突然変異体の原因が細胞壁タンパク質の生合成能にあることが明らかにされた。松本氏からは除草剤の作用機構についての話があり、ジェフェニルエマルス除草剤については光が必要だということは以前からわかっていたことと思いますが、突然変異体を用いた仕事でキサントフィルが必要だということ、別にクロロフィルを起す葉緑を散布して生じた白色イネもアルピナと同様の挙動（この除草剤に対して）を示すという話をされた。除草剤の作用機構への一つのアプローチかと思いますが、ついていきますと除草剤の効き方を含めて光のエネルギーの移行の問題にまで入っていくことになるかと思います。DCPAについては、解毒的なアルカリシルアミダーゼが確実に関与していることが突然変異体を使って明らかにされた。また除草剤アトラジンについてもトラモロコンで三つの解毒系がありそれぞれ単因子で支配されることが、アトラジン抵抗性のものがみられたなどの紹介があった。この点については遺伝子をやっているものか否か選抜す突然変異かという点で面白いことだと思う。そのほか大気汚染に関しての品種間差異や遺伝的支配の話があった。

これらを総括して突然変異体を使っての研究を整理してみると、遺伝的バックグラウンドが同じで一つだけの遺伝子が変わったものを材料として作用パターンやパラメータを比較できるという最大のメリットがある。突然変異体を使う研究のすすめ方としては一つには純粋に生理の研究、例えば物質の合成過程のどこを突然変異体がブロックしているかを追求する使い方がある。しかし育種の方がみると、そういった研究から出て来た結果を選抜の場合の基準などに使えるかどうかという問題が残る。もう一つの考え方は、突然変異体を利用して場合生理研究からえられた情報が役立つわけではないかと思う。その場合交配親として用いる上で遺伝子の表現型は同じでも作用機構が違っていわば使い方がちがってくるだろうと思われる。

更にもう一つの面として、現時点では遠き将来の問題かも知れないが生産形質に関しても、生理的な面からつっこんでいって、突然変異遺伝子の働き方がわかっていて、それを実際に突然変異を誘発し選抜しうる利用する場合に役立つのではないかと思われる。

木下：相対氏の話を要約すると、遺伝子の形質発現について突然変異形質を利用した一つのモデル実験である。穂の抽出前の短期間の低温により可視的な明確な差が現われる。また栽培条件でも窒素レベルの差により大きな影響が出ることなどで示された。

業者氏の構造は白い所ではプロトプラスタ、緑色化するとプラスタ構造に回復する。リポゾームは白い所には現れないが酵素系だけは残存している。またキサンタの場合にもやはり様々な条件で変化するという話だった。すなわち同一の遺伝子を保有していても形質表現される場合とされない場合がある。遺伝学の教科書ではこれを expressivity とか penetrance という概念で表われされ、あまり明確には記述されていない。これを相対氏は生理的に非常によく解釈されて、ある特定の期間における、ある温度条件がその酵素系の形質発現を支配する事を指摘された。すべての遺伝形質でこの様に解析できるわけではなく
更に複雑なパターンをとる場合も多いと考えられる。

最後に放射線育種場の方から大麦、イネ、大豆、野菜、木本作物等について突然変異形質の紹介があった。これらの遺伝様式を調べる必要があるだろう。イネの連鎖地図の研究ではこの様な突然変異形質の遺伝子分析を行っている。イネ、大麦では連鎖地図がよく出来来てたので、今後はこの突然変異形質の登録だけにとどめないで遺伝子を解析し、少なくともどの関連群の遺伝子であるか知る様にし、それを基礎にして生理学的あるいは遺伝育種学的な研究に使うならば更に有効であろう。これには遺伝形質の保存と同様に組織的な研究体制を整える事が必要と考えられる。

大畑根：それでは山口氏の講演から討論に入りたい。

坂：細葉型では葉面積当たりの光合成活性は2倍ほど高いことを強調されていたが、これに乾物重、全窒素、蛋白態窒素をみるとあやしくなってくる。特に蛋白態窒素では、むしろ細葉型が低くなる結果がみられる。この点について伺いたい。また葉面積当たりの光合成速度と収量とは相関がないのではないか。私のところでも細葉の突然変異体があるが、葉面積当たりの光合成速度などは高いが蛋白あるいは収量でみると低い。

山口：一応比較のために葉面積当たりで測ってみた。多分細葉型では細胞の大きさは小さくなっているが葉緑体数は変わらないのではないかと考えている。

中島：細葉系統の収量性について伺いたい。

渡辺：細葉型突然変異体のNF-1は一種の粒数が非常に少ない。そこで明徳5号という品種を母体にして交配し、冊系G系統を作り、これで1種の粒数が倍増になった。収量を栽培密度、播種密度を多くするという観点から調べてみたが、一次枝梗数、一次枝梗当たりの着粒数が明徳5号のように多くならずササニシキ程度にしかならなかった。したがって、現在の段階ではササニシキ程度の収量しかあげることができない。普通の栽培ではササニシキよりも収量は低かった。生育中に窒素量を増すと、若葉は伸ばすが登熟過程が低下するという議論があるが、この時期に施肥を増していくとササニシキは収量が低下する。しかし細葉系統は収量が増加してくれるという特異性がある。このため、いろいろの品種を交配して地域平均収量1,000kgを目標にして系統を作っていきたい。このためには細葉でなければならないと考えてやっている。

佐々木：山口氏の突然変異率の図1でパーシャルアシナブンスの突然変異体K648が高い値を示した。この比率の高いものからの突然変異体はそうでないものからの突然変異体と同じようなものでしょうか。もし半数体の照射で出てくる突然変異体の利用度が高いなら花粉の照射でそのような突然変異が得られる易いと考えられるか。

山口：多分花粉照射の方が突然変異率は高くなると思うが、染色体の欠失的なものの割合が多くなるのではないかと考える。

佐々木：そういう欠失によるものが生理研究等に利用される場合もあるのではないか。

大畑根：次に角田氏の講演について。脂肪酸生成合成の進化の図で地衣、羊歯類脂肪酸のCの数が急に減ってくるが、系統発生的にどのような意味をもつか。

角田：この図で実際は不飽和の脂肪酸の推移を示したもので不飽和の数が6まで達し3に落ちたのが主な意味がある。薬薬が陸上にあがって来た段階で、不飽和数がどういうわ
けでおちたのかということであるが多分、陸上での適応が関係していたのではないかとRöbbelen はいっている。

大曾根：進化における不飽和酸の意味は何か。

角田：一つは不飽和になれば油が軟かくなるから低温のところに良い。貯蔵体でも構造脂質として細胞壁を形成している場合も同じ傾向だといえるもう一つは光合成との関係をいう人がいる。

大曾根：子葉がグリーンのものは不飽和脂肪酸が多いというがそれはどんな意味をもつか。

角田：不飽和のすすみ過ぎたものを少しとめて、リノール酸の多いものを作りたい場合に、それが生育と関係に出来るかどうか、特に大豆、ナタネのように種子の時期にグリーンを絶対するものでどの程度可能かはよくわからない。突然変異体を作る一方、自然界に存在するものをサーベイして、不飽和脂肪酸形成と生育との関係を今後調べを見てみたい。

大曾根：エリシノ酸の方は零％のものが出てくるが、不飽和脂肪酸のリノレン酸に零％のものがいないのはリノレン酸は必ず必要だということか。

角田：ベニバナ、落花生は零％だが、ナタネ、アマ、大豆みたいに子葉をたべる種子の場合はリノレン酸が零％のものはみつかっていない。零に出来ないのかあるいは零にした時発の稔実、発芽にどう関係してくれるかということは興味のある問題だ。ドイツで突然変異の実験をやったが、零％のものは出て来なかった。

大曾根：エリシノ酸の方はあってもなくてもよく、だから零％も出るのだといってよかったか。

角田：そのように思う。しかし本当は種子の発芽とか初期生育にリノレン酸が関係があるかどうかの正確なところはわからない。通常の栽培条件下では支障がない。

倉石：脂肪酸形成合成の進化の図で藻類といっても紅藻、褐藻、緑藻は互いに非常に違い関係にある。またキノコの仲間の菌類はこの中でどのような配置を示すのか。

角田：Wagner & Pohl が 1966 年にデータを出し、Röbbelen がそれを引用したものがこの図である。興味があったら原文本をみいていただけたらと思う。

中島：脂肪酸形成の基本型の図でプラシン型には A, B, C の基本ゲノムをもつ種とそれぞれ相互の複数倍体の種があるが、このようなゲノム構成との関連で、図の脂肪酸形成のプラシン型をさらに細分化できないか。

角田：十字花科の系統発生との関係で言ったもので、他のアブラナ科植物の大半はプラシン型をとる。プラシンの基本種三つもその複数倍体も全部この型である。ただアブラナ科を広範的にみると若干のものはエリシノ酸を形成しない。

松中：現在雨宮氏が主として化学物質を使って油を軟かくし耐寒性を増すことにねらいをつけて研究しているが、突然変異の方からいうと合成経路のどこかを切るという方向でいけるか。

角田：合成経路のどこか切れば炭素鎖が短くなり不飽和度が少なくなると思う。

大曾根：次に倉石氏の講演について討論願いたい。
山下：オオムギで子葉鞘の上の方ではハイドロオキシンプロリンの比率が低く、かつ渦で変らない。下の方だけが変っているということは、下の方が早くエイジングがすすむと考えられる。IAAが少ない場合には早くエイジングすると考えてよい。

倉石：渦の場合、オーキンが有効に効くのでオーキシンを処理した上で細胞壁蛋白質（ハイドロオキシンプロリンが多く含む蛋白質）がどれだけ多くなるかを測りたいと思っていている。これを測れば今の質問の答えがでると思う。

大島根：渦、並の違いによっていろいろの形質が変わってくることは、多面発現の例としつよく教科書など出てくるが、全部オーキシンの作用と考えてよいか。

倉石：オーキシン自体がいろいろな生理作用を示し、また間接的にオーキシンの生産に関係する何かがあるとすれば多面的であってもよい。その辺はどうなっているかこれら特定の、渦の場合、それをとってくるとオーキシンが効かなくなる。それが渦が生産性が高いという説拠だろうと思う。始めから終わりまでオーキシンあるいはGAが少ないとか、細胞壁蛋白質も変わらないというので実用的によくないのではないか。昨日、矮化植物を選ぶ場合の生理的な面でのメルクマールはいかつかとの質問があったが、その一つとして光合成が考えられる。しかし、それは、C4やC3植物等の問題で問題がある。もう一つは、細胞の中に入りCO₂濃度がどの程度であるかを測ることが考えられる。これはについて、いくつかの植物で差がみられている。このようなことを追いかけてみること、葉の寿命、転流など、今までそれほど気付かなかった問題で生理的に面白いものがメルクマールになっていくことも予想される。

奥野：矮性突然変異遺伝子に限らず突然変異遺伝子の形質発現を考える場合に、最初のアプローチの仕方としてホルモンのぶっかけ試験をやる。そのぶっかけ試験の結果にとづいて、体のいずれかのホルモンをぶっかけてみるというやり方をとる。例えばよく変性遺伝子とジペレリンの問題を考えた場合、変性遺伝子の中でジペレリン反応したりしなかったりするものがある。この時、ジペレリン以外で何かホルモン的なものが関係しているのではないかということ、ジペレリン含量が高くてもその体内でジペレリンが不活性化されるような、ジペレリン阻害機構をもつ矮性品種があるのではないかということを考えられる。私が試験をやった時、変異体の中でジペレリン反応しないでサイクリックAMPを与えたらある濃度で反応するものがあった。サイクリックAMPがジペレリンの誘発剤のような形で効いているというようなことを読んだ事があるが、このような形質発現の問題を考える場合はホルモンの関係で何かコメントをいただけないか。

倉石：ジペレリンが体内に多いのに矮性になっているという場合がよくある。一般的によく起きる現象としてジペレリンを非常に早くグルコースにすることがある。オーキシンの場合アスパラギン酸と結合してアミノアスパラギンを作るものがあるが、このような形になると、液胞の中に入ってしまって二度と出てくるため、生体のホルモン量は減るという現象が起こる。ただ抽出だけではわからない、かといって液胞とグルコースどううまく分けるか方法がない。またサイクリックAMPとジペレリンとの関係は、最近ではそれぞれ独立したものであるという考え方が強いようだ。

大島根：次に松中氏の講演に移りたい。
山下：雄性不稔に除草剤抵抗性を連鎖させて雄性不稔の個体だけを残すということが望まれている。そのためには除草剤感受性が遺伝的に優性でなければ具合が悪い。除草剤の種類によって優性で感受性になるという例はあるか。

松中：今のところない。そういう実験をやっているが労性である。

角田：アトラジンに対する感受性がC₃とC₄で二つに分かれるということを、7〜8年前カールソンが言っていました。その理由および現在もそのようなかどうかをききたい。

松中：C₃C₄の違いでなくて、彼が扱ったC₄植物が特殊な解毒酵素を偶然にもっていたと解釈する方がよいように思う。同じC₄植物でもトモロコシのGT122アイソジェニックな植物の間には、アトラジンに対して抵抗性と感受性がある。その場合にグルタチオンをアトラジンにくっつける酵素（グルタチオンS-トランスフェラーゼ）の活性が1.63と0.03という値のちがいがあり、そういった遺伝的なちがいが効いている。

大曾根：次に相賀氏の講演に移りたい。

鶴岡：温度感受性を示す系統が全体の菜緑素変異の中の何％をしめるのかというところ、それが突然変異誘発物質の処理の強さに関係しているかどうかを伺いたい。

相賀：どの辺の％かは気にしていないが、突然変異誘発物質の種類には関係ない。

長谷川：私のところでは菜緑素突然変異の検定を、昼25〜27℃、夜20℃の条件で行なっているが、時折葉緑によって色の異なる突然変異体がみとめられる。この場合昼夜の高低温度が遺伝子発現にどのように影響していると考えられるか。

相賀：ν₁、ν₂、ν₃は菜緑素の発現、菜緑素の生合成ということに関連してどのようなメカニズムで白葉になるのかわからないが、一つの考え方は菜緑素合成系そのものはやられなくておらず菜緑体の発育初期期に白葉化、緑葉化を方向づけるようにと思われる。その時期に温度が敏感に効いてその時期を高温で経過すれば緑葉になるように方向づける。また、低温で経過すれば白葉になるように方行づけられる。代謝的なものでなく方向性を定めるトリガーのような機能を温度がもっていると考えている。

小野沢：ビリディスレベルの菜緑素突然変異体の生死は菜緑素量のみに依存するのか、あるいはほかの要因があるとか考えられるか。

相賀：第1にビリディスが温度感受性をもっていて、低温でアルピノまたはキサンダ様になる場合致死となる。生育途中で高温になりビリディスが正常葉に近くなる場合生存し得ることがある。従って菜緑素量に依存しているように思われる。

第2に緑葉で多数報告があるように、光合成系の特定部位が遺伝的に欠損し、菜緑素量に依存せずに致死となる例はイネについてはまだ見つかっていない。

大曾根：放射線育種体で多くの突然変異体を紹介されたがこういう突然変異体は出ないかとか、もう少し詳しく聞きたいといいところはないか。

平野：実用形質でないものが多くの説明されたが、育種家は実用形質を非常に期待している。耐病性育種をやっている関係で大変の白痢病抵抗性についての放射線育種の可能性があるかどうか聞きたい。
山下：ビール麦のアズマギールデンに耐病性突然変異が3系統得られている。これには葉にフレッケンを伴う欠点があるが、バックグラウンドを変えてやると少なくすることが出来ると主張している。これが育種に役立つかどうかは将来の問題だが、この種の耐病性突然変異は可能だということは明らかだと思う。

渡辺：小麦にモチは出ないこと。

山下：突然変異体の種子の中まで調べていないのでわからない。

大澤根：6条大麦から2条大麦は出来ないか。

山下：その例は今までにならない。

相賀：色素の突然変異体で穂穀素bだけでaがない突然変異体があるかどうか。またカドノイドのない突然変異体があるか。

山下：薬の色の変わったものを選抜しているだけで、内容については調べていない。しかし穂穀素a、bの相対顕度の非常に多いものは、エンドウなどでもドイツのGottschalkなどによってみられているので、その中には色々あるのではないかと思う。

相賀：穂穀素の比較的多くてbがないケースは非常に多いだろう。しかし、aがなくてbだけだというのは今までないと思うがどうか。

山下：よくわからない。

山口：病斑の出る突然変異体は本当に病原菌がないか。

山下：調べていないから、外国の例では無菌的にやっても病斑が出るという報告も聞いているので、生理的病斑ではないかと推測している。

相賀：穂穀素生合成系が異常になり、ボルフィリンの蓄積がおこって光感受性になり結果においては褐斑になる場合があるのではないか。この場合紫外線照射により褐斑部位が赤い発光を発することで区別出来ると思われる。

平野：農林8号の突然変異体の中に渡辺氏のNF-1と同じような細葉で分けつ数が多いのがあったが、これを密植して肥料を多くやったとき収量がどうなるか興味深い。また藤坂で10年前フジミリの無分けつ穂を作ったのが不穏が多く、交配して穂数を増やすとしたのがものにならなかった。紹介のあった少分けつ穂は直播栽培を考えると長範で、穂数が良ければ役立つであろう。これからも、そういう材料があったら捨てないで残しておいてもらいたい。

佐々木：お聞きのように突然変異体は生物の研究に有効に利用できることが示された。今後より多くの突然変異体を作り、その遺伝的バックグラウンドをはっきりさせて、共同して情報交換しあっていく必要があると思われる。最後に松尾先生にしみるべきお願いしたい。

松尾：放射線育種場から多くの突然変異系統をみせていただいたが、保存が大変だろう。組織的な保存の方法など今から考えておかなければならないと思う。

佐々木：これで終わります。講師、御会議の皆様どうも有難うございました。
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