Induction of Mutation by the Treatment of Fertilized Egg Cell with N-methyl-IV-nitrosourea in Rice

Satoh, Hikaru
Laboratory of Plant Breeding, Faculty of Agriculture, Kyushu University

Omura, Takeshi
Laboratory of Plant Breeding, Faculty of Agriculture, Kyushu University

https://doi.org/10.5109/23706

出版情報：九州大学大学院農学研究院紀要. 24 (2/3), pp.165-174, 1979-11. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., *24* (2·3), *165-174 (1979)*

Induction of Mutation by the Treatment of Fertilized Egg Cell with N-methyl-IV-nitrosourea in Rice

Hikaru Satoh and Takeshi Omura

Laboratory of Plant Breeding, Faculty of Agriculture,
Kyushu University 46-01, Fukuoka 812

(Received August 11, 1979)

In order to increase the efficiency of induction of mutations by chemical mutagens, the fertilized egg cells at 6 hours after flowering were treated with MNU. The frequency of mutations and the efficiency in the treatment of fertilized egg cell greatly exceeded those in the treatment of dry seeds. The possible concentration of MNU applied on the fertilized egg cell was estimated less than 1.0 mM.

INTRODUCTION

Mutation breeding is one of the most effective methods for improving crop plants. On the standpoint of mutation breeding it should be taken into consideration to increase the frequency of mutations and to control the spectrum of mutations.

Many kinds of chemicals, now, are used as an effective mutagen to induce a large number of gene mutations with less chromosomal aberrations compared with radiations (Heiner et al., 1960; Froese-Gertzen *et al.*, 1964; Künzel, 1971). However, the application of the chemical mutagens have been restricted to almost only the dry seeds because they are toxic to growing plants.

Recently, it has been made clear in higher plants as well as microorganisms that the mutagenic ability of chemicals relates with the specific stage of DNA synthetic cycle (Yamaguchi, 1972; Yamaguchi and Matsubayashi, 1973; Nishimura and Futsuhara, 1976). However, dry seeds of rice and barley are mostly in G_1 (Yamaguchi, 1969, 1972). In order to enhance the mutagenic ability of chemical mutagens, therefore, the preferential application of them at the specific stage of the cell cycle and the suitable method for treating the growing cell of higher plants should be required.

The single cell stage of the embryogenesis seems to be the most desirable to the induction of mutations because no diplontic selection and a maximum size of mutated sector are expected in the treatment at this stage.

From these viewpoints, we tried to treat the fertilized egg cell of rice at the single cell stage with *N*-methyl-*N*-nitrosourea (MNU), which is one of the most effective mutagens on the bacteria (Hince and Neale, 1974 a, b, 1975) and is able to induced mutation in higher plants (Muller, 1965; Michaelis *et al.*, 1965; Gichner *et al.*, 1968). In this paper the efficiency of the induction of mutations by the treatment of fertilized egg cells in rice is compared with

that by the treatment of dry seeds.

MATERIALS AND METHODS

In a series of experiments, a paddy rice variety "Kinmaze" was used as a material, and *N*-methyl-*N*-nitrosourea (MNU) gained by Maruwaka Kagaku as a mutagen.

1. Sensitivity of dry seed and soaked seed to MNU

To estimate. the suitable concentration of MNU applying to growing plant of rice, sensitivities of dry seed and soaked seed to MNU were compared. One hundred enriched dry seeds of Kinmaze with 13 % moisture and one hundred seeds soaked in distilled water for 60 hours at 25°C in the dark were immersed in the MNU solutions for 2 hours at 25°C in the dark and followed by washing for 24 hours in running tap water. The concentrations used were 30, 40 and 50mM on the dry seeds, and 2.5, 5.0, 7.5 and 10.0 mM on the soaked seeds. After treatments they were immediately grown in the incubator controlled at 25°C in the light for 13 days.

Sensitivities of both dry and soaked seeds to MNU were evaluated on the bases of the germination percentage, the survival rate and reduction of seedling height and root elongation at 13 days after treatments. The germination was judged by means of emergence of the first leaf from the coleoptile in this experiment.

2. Treating the fertilized egg cells with MNU

Fertilized egg cells of Kinmaze at 6 hours after flowering were treated by the panicle dipping-method. The procedures are given as follows : In the evening of one day before treatment, the plants were transplanted from the field to pots and spikelets already opened were cut out. To control the developmental stage of fertilized egg cells as same as possible, only the spikelets flowered within one hour from 10 : 30 untill 11 : 30 were used and unopened spikelets were also cut out. At 6 hours after flowering, the panicles with these spikelets were immersed in 1.0 to 4. 0 mM MNU solutions for 1 to 3 hours at about 24°C under the dimlight condition. The solutions were controlled pH at about 4.8. The panicles were washed immediately after the treatment for 24 hours in running tap water. More than two hundred spikelets were treated in each treatment. The panicles immersed in distilled water for 3 hours and followed by washing for 24 hours in running tap water were used as a control. M_1 seeds set on the treated panicles were harvested at about 40 days after treatments.

In the next year, the M_1 seeds were sown on the small seed beds and grown in the green house for about 40 days until1 they were transplanted into the paddy field in the Kyushu University Farm. After ripening, the longer two to five M_1 panicles per each M_1 plant were harvested. The biological effects of MNU on the fertilized egg cell were evaluated by means of following criteria: M_1 seed setting per treated spikelets, maturity of their seeds, germination percentage of M_1 seeds, seedling height at 15 days after sowing, nurs-

ery survival of M_1 seedlings at the time of transplanting, which is the ratio of transplanted M_1 seedlings to M_1 seeds sown, field survival of M_1 plants at the time of harvesting, which is the ratio of M_1 plants survived until1 the time of harvesting to transplanted M_1 seedlings, the number of sterile M, plants per 100 M_1 plants and final survivals. Here, the sterile M_1 plant means one having at least a sterile panicle showing fertility less than 80 %, and the final survival means the number of M_1 plants setting more than 30 M_2 seeds on their three panicles per 100 M_1 plants. The frequency of mutations in M_2 was estimated by the number of chlorophyll mutations per 100 M_1 plants. In addition to the frequency of chlorophyll mutations, the efficiency of induction of mutations, which is the number of chlorophyll mutations per 100 M_1 seeds sown, was employed to compare the effectiveness of induction of mutation among treatments.

For the comparison with the above mentioned treatment, dry seeds were treated with MNU by following conditions: Four hundred enriched dry seeds were immersed in solutions of 5.0, 10.0, 15.0 and 20.0 mM MNU for 4 hours at 25°C in the dark. The solutions were adjusted pH at 4. 8. After treatments, they were washed in running tap water for 24 hours. Four hundred enriched dry seeds were immersed in distilled water controlled pH at 4.8 for 4 hours and followed by washing for 24 hours in running tap water to use as a control.

RESULTS

1. Sensitivity of dry seed and soaked seed to MNU

Biological effects of the treatments are shown in Fig. 1. Although injuries at seedling stage of M_1 increased with the concentration of MNU both on the dry seeds and on the soaked seeds, the latter were more injured, *even the* concentration of MNU was below one tenth of the former. For example, the concentration inducing 50 % lethality (LC 50) was estimated at about 5. 0 mM in the soaked seeds, whereas it was about 50mM in the dry seeds. Furthermore, the concentration inducing 50 % growth reduction of seedling (RC 50) was also estimated at about 2.5 mM and 30mM in the soaked seeds and the dry ones, respectively.

On the other hand, MNU inhibited rooting and root elongation more strongly than germination. Especially, the growth of crown root was remarkably depressed in both treated seeds. The inhibition of the growth of root on the treated seeds seems to be one of the major reasons for the lethality of treated seeds.

These results indicate that the concentration of MNU applying on the growing cell is preferable to restrict below 5. 0 mM. In the panicle dipping treatment, therefore, MNU solutions under 4. 0 mM were used.

2. Treating the fertilized egg cells with MNU

M_1 injury

Seed setting and the maturity of harvested M_1 seeds are shown in Table

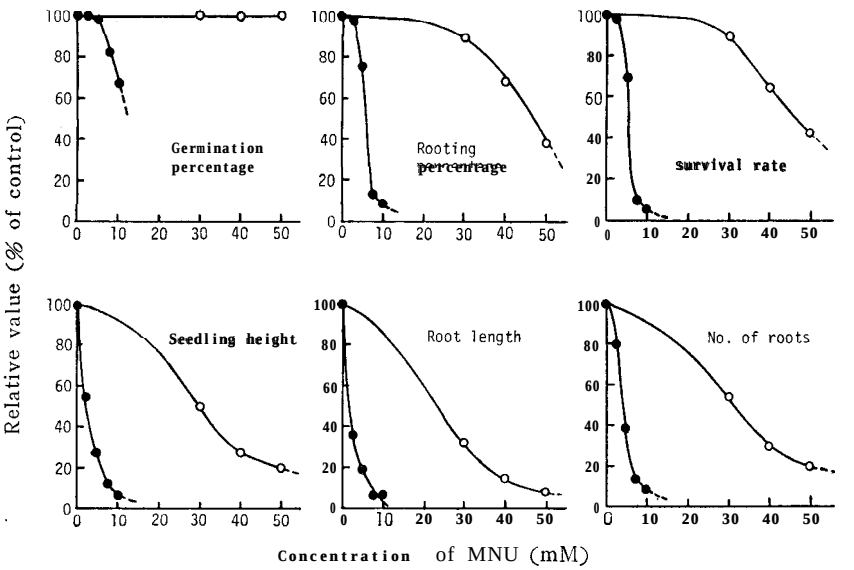

Fig. 1. Sensitivity of dry seeds and seeds soaked in distilled water for 60 hours at *25°C* in the dark to MNU. —○—, dry seeds; — ● --, soaked seeds.

Table 1. M_1 seed setting and its maturity after the treatment of fertilized egg cells with MNU at 6 hours after flowering

Treatment		No. of treated spikelets	Harvested seeds		Maturity of harvested seeds
Conc. (mM)	Period (hr)		Number	%	
1.0	2	230	224	97	good
	3	334	325	97	a little wrong
1.5	2	329	315	95	a little wrong
	3	324	307	94	wrong
2.0	2	301	222	95	wrong
	3			89	very wrong
3.0	1			74	very wrong
Water	3	426	387	91	good

Treatment with 4.0 mM for 1 hour was excluded from the table because of very few seed settings.

1. The treatment with 4. 0 mM for 1 hour induced necrosis on the soaked panicles, and resulted in few seed setting. The treatment with 3mM for 1 hour also induced the necrosis and reduced the seed setting to about 70 % of control, in addition, most of the harvested seeds were immature. No necrosis was observed by the concentration less than 2. 0 mM, whereas about 20% of harvested seeds were immature even by 1.0 mM for 3 hours.

It was found from these results that the concentration below 2. 0 mM was apropriate to the method, so panicles were dipped for inducing mutations at 6 hours after flowering in 1. 0 mM to 2. 0 mM MNU solutions for 2 and 3

hours.

The germination percentage, seedling height and nursery survival of M_1 seedlings were, as shown in Table 2, affected by the treatment, especially the nursery survival was reduced against the concentration of MNU. The nursery survivals in the treatments with *2.0* mM for *2* and 3 hours were *54* and 45 %, respectively. It was also affected by the duration of dipping, for example at 1.0 mM, being 21 % lower in the dipping for 3 hours than that for 2 hours. Field survival was not so much different among conditions of treatment (Table 3). However, the number of sterile M_1 plants increased drastically with the concentration. On the other hand, the treatment with 1.5 mM for 3 hours induced fewer sterile M_1 plant than that with 2.0 mM for 2 hours, though the dosage of treatment (concentration x duration of treatment) of the former was larger than the latter. This situation was almost the same as the other M_1 injuries. Consequently, it seems that the toxic effects of MNU to the fertilized egg cell are more strongly caused by the concentration than by the duration of treatment. In the treatments of fertilized egg cells, perfectly sterile M_1 plant was caused more frequently compared with the treatments of dry seeds as shown later. As well as the lethality during the growth of M_1, appearance of perfectly sterile M_1 plants reduced the number of M_2 lines cultured. Considering this point, final survival was employed to evaluate finally

Table 2. Injuries at seedling stage of M, derived from the treatment of fertilized egg cells with MNU at 6 hours after flowering

Treatment		No. of M_1 seeds sown	Germination (%)	Seedling height (%)	Nursery survival (%)
Conc. (mM)	Period (hr)				
1.0	2	224	99	81	
1.5	2				98
2.0	2	313 297	72 79	66 53	63 54
1.5	3	325	98	64	77
2.0	33	464 216	87 74	65	45 54
Water	3	387	99	100	99

Table 3. Field survival and sterility of M_1 derived from the treatment of fertilized egg cells with MNU at 6 hours after flowering

Treatment		No. of transplanted M_1 plants	Field survival		Sterile M_1 plant (%)	Perfectly sterile M_1 plant (%)	Final survival	
Conc. (mM)	Period (hr)		Number	%			Number	%
1.0	2	219	179	82	69	24	136	61
1.5	2	197	123	62	82	47	65	21
2.0	2	160	132	83	98	92	10	3
1.0		250	174				136	
1.5	3			70	74	22	50	42
2.0	33	208 117	102 105	87 50	85 97	51 59	43	239
Water	3	350	350	100	0	0	350	100

the damage of M_1 induced by MNU. It was remarkably decreased with the concentration, showing below 50 % in the treatment with 1.0 mM for 3 hours and only 3 and 9 % in those with 2.0 mM for 2 and 3 hours respectively.

The injury in M, of treating dry seeds was not necessarily as same as that of fertilized egg cells. Though the seedling height was decreased against the concentration, no lethality of seedling was observed (Table 4). Field survival reduced to under 70% by the treatment with 15.0 mM MNU, whereas by that with 10.0 mM it was more than 90 % (Table 5). Sterile M_1 plants ranged from 6 to 47% by the treatments and the values were less than those in the treatments of fertilized egg cells. Final survivals, 92 to 55 %, were considerably higher than those of 61 to 3% in the treatments of fertilized egg cells. It is caused by the fact that there is no lethality of M_1 seedling and no perfectly sterile M_1 plant in the treatments of dry seeds.

Frequency of chlorophyll mutations in M_2

Frequencies of chlorophyll mutations in M_2 of both the treatments of fertilized egg cells and dry seeds are shown in Tables 6 and 7, respectively. In the treatments of dry seeds, number of chlorophyll mutations per 100 M_1 plants was 32 at maximum in the treatment with 20.0 mM for 4 hours. On the other hand, the treatments of fertilized egg cells induced from 40 to 70 chlorophyll mutations per 100 M_1 plants.

On the treatment inducing mutations for plant breeding, however, it should be required to decrease the lethality in M_1 as well as to enhance the frequency of mutations in M_2. In this respect, the efficiency of induction of mutations was employed as a criterion to evaluate the effectiveness of the

Table 4. Injuries at seedling stage of M_1 derived from the treatment of dry seeds with MNU

Treatment		No of M_1 seeds sown	Germination (%)	Seedling height (%)	Nursery survival (%)
Conc. (mM)	Period (hr)				
5.0	4			85	99
10.0	4	400 400	100 100	80	99
15: 0	4	400	100	56	98
20.0	4	400	100	48	99
Water	4	400	100	100	100

Table 5. Field survival and sterility of M_1 derived from the treatment of dry seeds with MNU

Treatment		No. of transplanted M_1 plants	Field survival		Sterile M_1 plant (%)	Perfectly sterile M_1 plant (%)	Final survival	
Conc. (mM)	Period (hr)		Number	%			Number	%
5.0	4	396	368	93	6	0	368	92
10.0	4	397	358	90	27	0	358	90
15.0		392	263	67	45	0	263	66
20.0	4	395	222	56	47	1	219	55
Water	4	300	300	100	0	0	300	100

Table 6. Frequency of chlorophyll mutations and efficiency of the treatment of fertilized egg cells with MNU at 6 hours after flowering

Treatment		No. of M_1 seeds sown (a)	No. of tested M_2 lines (b)	No. of chlorophyll mutations (c)	Frequency (c/b x 100)	Efficiency (c/a x 100)
Conc. (mM)	Period (hr)					
1.0		224	136	67		30
1.5	2				49	
2.0	21	313 297	65 10	42 7	64 70	13 2
1.0	3	325	136	64	47	20
1.5	3					
2.0	3	464 216	43 50	29 17	40 58	13 4
Water	3	350	350	0	0	0

Table 7. Frequency of chlorophyll mutations and efficiency of the treatment of dry seeds with MNU

Treatment		No. of M, seeds sown (a)	No. of tested M_2 lines (b)	No. of chlorophyll mutations (c)	Frequency (c/b x 100)	Efficiency (c/a x 100)
Conc. (mM)	Period (hr)					
5.0	4	400	368	73	9	8
10. 0	4	400	358	60	20	18
15.0	4	400	263		23	15
20.0		400	219	69	32	17
Water	4	300	300	0	0	0

treatment. In the treatments of fertilized egg cells, the maximum value of 30 % was given at 1.0 mM for 2 hours, whereas in the treatments of dry seeds it was only 18 % at 10. 0 mM for 4 hours and the concentration more than 10.0 mM didn't increase it though increased the frequency of chlorophyll mutations.

DISCUSSION

Only a few trial on the application of chemical mutagens to growing plants has been reported (Bowen, 1965; Nybom and Koch, 1965; Ferrary, 1965; Grober, 1966; Onozawa, 1972; Ashri and Levy, 1974), although many studies were carried out for the application to dry seeds. This is due to the technical difficulty of treating fresh organs of growing plants with chemical mutagens and comparatively low frequency of mutations induced.

On the other hand, the corpus initial cell group of the rice embryo, which leads to the main stem, consists of about 5 or 6 cells in maximum (Osone, 1963) and it always leads to the chimera formation on the treatment of dry seeds (Yamaguchi, 1962). The chimera formation indicates the small size of mutated sector and as the results the segregation ratio of mutants reduce. In addition, the frequency of mutations would be decreased by the diplontic selection between mutated and unmutated cells in the meristem, as reported by Gaul (1961, 1964). Therefore, the monocell stage of embryo, that is the

fertilized egg cell, seems to be the most desirable stage of treatment (Mericle and Mericle, 1962). However, growing plants are more sensitive to radiation and chemical mutagens (Yamashita 1967; Osone and Mikaelsen, 1970). Further, most of chemical mutagens are effective carcinogens and they are highly toxic to plants as well as animals and microorganisms. Therefore, to treat the fertilized egg cells in rice with chemical mutagens, the effective method of treatment and the employment of the effective mutagen must be established.

The panicle dipping method was applied in this study because this method is easier to treat the fertilized egg cells and less toxic than such methods as the absorption of mutagen through the root and the injection into the internode. The panicle dipping method seems to be very effective (Table 1). Onozawa (1972) reported that the panicle dipping method was useful to treat the developing embryo in rice and barley with chemical mutagens. The concentration of MNU applied on the fertilized egg cells was decided on the bases of the results that the appropriate concentration of MNU on the treatment of soaked seeds was below 5. 0 mM. Even in the treatment with 3.0 mM MNU for 1 hour, however, necrosis was induced. This result suggests that the fertilized egg cell is more sensitive to MNU than the soaked (germinating) seeds. From the results shown in Table 2, the possible concentration applied on the fertilized egg cell of rice is estimated at or below 2. 0 mM.

The treatment of dry seeds is characterized by the growth reduction of M_1 seedling and the high lethality of transplanted M_1 plants (Tables 4 and 5). It may be caused by the growth reduction of roots shown in the first experiment (Fig. 1). On the other hand, the treatment of fertilized egg cells is characterized by the reduction of germination percentage of M_1 seeds and nursery survival of M_1 seedlings, and the induction of a large number of sterile M_1 plants (Tables 2 and 3). The loss of M_1 at seedling stage may be caused by the abnormality of the organization of embryo since the treatments are carried out before the embryogenesis commences. On the other hand, it is obscure how the high frequency of sterile M_1 plant is brought by these treatments, but it may be caused by occurrence of no diplontic selection in the materials treated at monocell stage.

The number of sterile M_1 plants was employed to estimate the effect of MNU on the fertility of M_1 plants in this experiment, though the fertility of M_1 population has been employed usually. On the treatment with MNU, both the number of sterile M_1 plants per 100 M_1 plants and the fertility of M_1 population highly correlated with the frequency of chlorophyll mutations in M_2 and their correlation coefficients are almost the same (Satoh, unpublished). Moreover, the number of sterile M_1 plants can be examined more easily than the fertility of M_1 population. This is the reason why the number of sterile M_1 plants per 100 M_1 plants is employed in this experiment instead of the fertility of M_1 population.

The frequency of chlorophyll mutations induced by the treatment of fertilized egg cells is clearly higher than that of dry seeds. It will be hoped in mutation breeding to increase the frequency of mutations in M_2 with less

lethality in M_1. A positive correlation generally exists between the damage in M_1 and the frequency of mutations in M_2 (Sato, 1966). However, it changes with the kind of mutagen and the method of treatment (Yamagata, 1966; Yamagata and Tanisaka 1977; Gichner *et al.*, 1968). It is, therefore, difficult to compare the effectiveness among different treatments. Considering this point, the efficiency of induction of mutations was employed in this experiment. In the treatments of dry seeds, it reached already a maximum value of 18 % at 10 mM for 4 hours, though the frequency of chlorophyll mutations increased with concentration of MNU (Table 7). On the other hand, in the treatments of fertilized egg cells, it was decreased with increasing the concentration of MNU applied (Table 6). Therefore, the efficiency of induction of mutations does not always agree with the increase of the frequency of mutations. The maximum value of the efficiency, 30 %, was obtained at 1.0 mM for 2 hours, though the frequency of chlorophyll mutations was not at maximum. This result shows that an appropriate concentration of MNU applied on the fertilized egg cell is below 1. 0 mM. It is finally concluded from these values on the efficiency that the treatment of fertilized egg cell with MNU is clearly effective in comparison with that of dry seeds.

REFERENCES

Ashri, A. and A. Levy 1974 Mutation yields and types obtained in peanuts, *A. hypoguea*, by treating mature seeds with ethyl methane sulfonate and gamma-rays, and developing embryos with ethyl methane sulfonate. *In* "Polyploidy and Induced Mutation in Plant Breeding," IAEA, Vienna, pp. 1-12

Bowen, H. J. M. 1965 Mutation in horticultural chrythanthemums. *In* "The Use of Induced Mutation in Plant Breeding," Pergamon Press, Oxford, pp. 695-700

Ferrary, R. 1965 Traitement de zygotes fécondés (orge) avec une substance mutagène. *In* "The Use of Induced Mutation in Plant Breeding," Pergamon Press, Oxford, pp. 293-297

Froese-Gertzen, E. E., C. F. Konzak, R. A. Nilan and R. E. Heiner 1964 The effect of ethyl methanesulfonate on the growth response, chromosome structure and mutation rate in barley. *Radiation Botany, 4: 61-69*

Gaul, H. 1961 Studies on diplontic selection after X-irradiation of barley seed. *In* "Proc. Symp. of the Effects of Ionizing Radiations in Seeds and its Significance for Crop Improvement. Karlsruhe, Germany (1960)," IAEA, Vienna, pp. 117-138

Gaul, H. 1964 Mutation in plant breeding. *Radiation Botany, 4: 155-232*

Gichner, T., H. Gaul and T. Omura 1968 The influence of post treatment washing and redrying of barley seeds on the mutagenic activity of N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea. *Radiation Botany, 8* : 499-507

Grober, K. 1966 Some results of mutation experiments in apples and currants. *In* "Induced Mutations and Their Utilization," Akademie-Verlag. Berlin, pp. 377-332

Heiner. R. E., C. F. Konzak, R. A. Nilan and R. R. Legault 1960 Diverse ratios of mutations to chromosome aberrations in barley treated with diethyl sulfate and gamma rays. *Proc.* N. *A. S.*, 46: 1215-1221

Hince, T. A. and S. Neale 1974 a Effect of N-nitroso-N-methylurea on viability and mutagenic response of repair-deficient strain of *Escherichia coli. Mutation Res., 22: 235-242*

Hince, T. A. and S. Neale 1974 b A comparison of mutagenic action of ethyl and methyl derivatives of nitrosamides and nitrosamidines on *Escherichia coli. Mutation Res.. 22:*

383-387

Hince, T. A. and S. Neale 1975 Effect of *Escherichia coli* growth rate on the number of mutations induced by N-methyl-N-nitrosourea. *Proc. Soc. Gen. Microbio.*, *2: 53*

Künzel, G. 1971 The ratio of chemically induced chromosome aberrations to gene mutations in barley. A critical study. *Mutation Res.. 12: 397-402*

Mericle, L. W. and R. P. Mericle 1962 Mutation induction by proembryo irradiation. *Radiation Botany,* 1: 195-202

Michaelis, A., J. Schöneich und R. Rieger 1965 Chromosomenaberration bei *Vicia* favu und Ascitestumororen der Maus nach Einwilkung von N-nitroso-N-methylharnstoff. Chromosome, 16: 101-123

Muller, A. J. 1965 Über den Zeitpunkt der Mutationsauslösung nach Einwilkung von N-nitroso-N-methylharnstoff auf quellende Samen von *Arabidopsis.* *Mut. Res., 2: 426-437*

Nishimura, S. and Y. Futsuhara 1976 Studies on the induction of mutations in rice by ethylene imine. III. Mutagenic activity of ethylene imine on the pre-soaked seeds. *Japan. J. Breed., 26: 99-109*

Nybom, H. and A. Koch 1965 Induced mutations and breeding methods in vegetatively propagated plants. *In* "The Use of Induced Mutation in Plant Breeding," Pergamon Press, Oxford, pp. 661-678

Onozawa. Y. 1972 Effects of chemical mutagens applied on growing plants. Gamma *Field Symp.*, 11: 61-72

Osone. K. 1963 Studies on the developing mechanism of mutated cells induced in irradiated rice seeds. *Japan. J. Breed., 13: 1-13*

Osone, K. and K. Mikaelsen 1970 Studies on embryo development and changes in sensitivity to gamma radiation and alkylating agent (MMS and EMS) in rice seed during soaking in water. *Japan. J. Breed., 20:* 187-196

Sato. H. 1966 Induction of mutation in rice by some chemicals. Gamma *Field Symp., 5:* 71–*88*

Yamagata. H. 1966 Mutagenic effects of several alkylating substances in rice with reference to the method of treatment. Gamma *Field Symp., 5* : 63-69

Yamagata, H. and T. Tanisaka 1977 Studies on the utility of artificial mutations in plant breeding. X. Effect of heat-shock treatment on radiosensitivity and mutation frequency in rice. *Japan. J. Breed.. 27 : 39-48*

Yamaguchi H. 1962 The chimera formation in an X_1 panicle after irradiation of dormant rice seed. *Radiation Botany, 2: 71-77*

Yamaguchi, H. 1969 The onset of DNA synthesis after soaking of rice seed as disclosed by the induction of mutations. *Radiation Botany, 9: 341-348*

Yamaguchi, H. 1972 The production of mutations by ionizing radiations and chemical agents in relation to the duplication of chromosomes. Gamma *Field Symp.*, 11: 29–42

Yamaguchi, H. and I. Matsubayashi 1973 Mutational response of rice seed to ethyl methane sulfonate and busulfan in relation to chromosome duplication. *Mutation Res., 17:* 191-197

Yamashita. A. 1967 Effect of acute and chronic gamma-ray irradiations on growing barley plants. Gamma *Field Symp., 6: 71-95*