Studies on the Utility of Artificial Mutations in Plant Breeding.

XIII. Mutagenicity of Several Alkylating Agents in Rice

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Five alkylating agents, ethylenimine (EI), ethyl methanesulfonate (EMS), N-nitroso-N-ethyl urea (NEUA), N-nitroso-N-methyl urea (NMUA) and N-nitroso-N-methyl urethane (NMUT), were compared on the mutagenic effectiveness and efficiency in a few agronomic characters of rice. The modes of dose-response of seedling height, survival rate of seedlings and seed fertility in M_1 generation were remarkably different with the different agents used: Methylating agents brought about more toxic effects on M_1 plants than ethylating agents. The data obtained in M_2 indicated that ethylating agents induced the mutations in chlorophyll, heading date and culm length more easily than methylating agents: In comparison of the highest frequency of chlorophyll mutants and variants in the two agronomic characters, NEUA ranked first, followed by EMS, EI, NMUA and NMUT in turn. The orders of the effectiveness and efficiency of mutagens were NEUA>NMUA>EI>NMUT>EMS and NEUA>EI>EMS>NMUA>NMUT, respectively. These results suggest that NEUA is one of the best mutagens in practical plant breeding. The frequency of variants in each of two agronomic characters was quite exactly proportional to that of chlorophyll mutations throughout all the agents applied.

Introduction

Mutation breeding has already been an essential tool for plant improvement, and not a little portion of its success seems to depend upon the appropriate choice and application of mutagen. Actually, a number of investigations for evaluating the mutagenic effect of mutagens have been conducted under diverse conditions of treatment, and a number of chemicals, especially of alkylating agents, have recently proved no less useful than ionizing radiations for inducing genetic variabilities in higher plants (GAUL *et al.* 1972, JANA and ROY 1975, KAUL and BHAN 1977, KONZAK *et al.* 1965, 1972, MIKAELSEN *et al.* 1971, NERKER 1977, NILAN *et al.* 1965, RAO 1977, RAO and NATARAJAN 1965). However, few reports have as yet concerned direct comparisons of mutagenic effects among the alkylating agents. Besides, the experimental results have mostly been discussed on the basis of chlorophyll-deficient mutations as appearing in M_2 seedlings. Therefore, it is considered necessary for actual breeding that the effects of various alkylating agents are evaluated also on the basis of mutations in agronomic characters.

The mutagenic effect of a mutagen, which is an index for the appropriate choice, can be evaluated in terms of mutagenic "effectiveness" and mutagenic "efficiency" (KONZAK *et al.* 1965). The former expresses the production of mutations per unit dose administered, while the latter does the proportion of induced mutations to other undesired biological changes simultaneously caused by the mutagen, such as lethality, growth impediment and sterility. Mutagenic effectiveness is of importance for evaluating the mutagenic capacity of a given mutagen, but the mutagens with high effectiveness (effective mutagens) are not necessarily those with high efficiency (efficient mutagens), because the effectiveness is determined independently of the undesired biological changes.

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Practically desired mutagens are, however, those that induce mutations in high frequency but cause low level of damages in treated generation. Therefore, the evaluation of a mutagen for usefulness should be made not only on its mutagenic effectiveness but also on its mutagenic efficiency.

From these considerations, five different alkylating agents were compared on the mutagenic effectiveness and efficiency in agronomic characters as well as chlorophyll characters of rice (*Oryza sativa* L.).

Materials and Methods

Unhulled seeds of a rice variety, Gimbōzu, were treated with seven degrees of concentrations of five alkylating agents, ethlenimine (EI), ethyl methanesulfonate (EMS), N-nitroso-N-ethyl urea (NEUA), N-nitroso-N-methyl urea (NMUA) and N-nitroso-N-methyl urethane (NMUT).

Seeds assorted by brine with 1.15 of specific gravity were used as the material. The seeds were presoaked in deionized water for 4 hrs., then treated at $22\pm 1^\circ\text{C}$ with freshly prepared mutagen solutions, whose volumes were adjusted each to 5 times of seeds. Treatments with nitroso compounds (NEUA, NMUA and NMUT) were made in 0.1 M phosphate buffer with pH 5, while those with EI and EMS were done in aqueous solution. The dose range used and the time of treatment were 2~80 mM and 3 hrs. for EI, 5~150 mM and 7 hrs. for EMS, 2~43 mM and 5 hrs. for NEUA, 0.5~30 mM and 5 hrs. for NMUA, 0.3~21 mM and 5 hrs. for NMUT, respectively. The seed soaked in deionized water or buffer solution were used as the control.

Treated seeds were washed for 18 hrs. in running water, then sown on germination trays in greenhouse and on nursery. In the M_1 generation, survival rate and seedling height at 14 days after sowing were examined for the seedlings germinated on the trays, and seed fertility after maturation of survival plants was scored for the plants grown in the field. Seedling height and seed fertility in each treatment were evaluated by mean values of 100 plants selected at random.

In each agent, four treatments of higher concentrations were examined in M_2 -progeny test. One hundred M_1 plants in principle were randomly selected from each of the treatments, then a panicle which headed first among tillers was taken from each of them. M_2 progenies of these selected panicles and those of the remaining ones were separately grown in nursery and in greenhouse, respectively, by panicle-row line method. When M_2 seedlings in both the nursery and the greenhouse were 10~15 days old, chlorophyll mutations were scored and classified after GUSTAFSSON (1940). Of the M_2 plants germinated in the nursery, those from three out of the four treatments in each agent were grown in the field and examined for heading date, culm length and seed fertility.

With respect to heading date and culm length, the plants outside the variation range of untreated population, determined at a critical rate of 0.01, were regarded as variants in the relevant character. Plants showing so much reduced seed-fertilities as to be clearly distinguished from the untreated plants were dealt with as sterile variants.

Results

(1) Biological damages in M_1 generation

Damages due to the treatments are presented in Fig. 1 by dose responses of the three characters, *i.e.* survival rate at seedling stage, seedling height and seed fertility. Data are expressed in percentage of the control.

In all the alkylating agents used, the damages significantly increased with higher concentration of agent but the modes of response were remarkably different among agents. In EI, all the three characters responded similarly to the intensity of treatment, but in other agents they responded differently from one another. When compared at the same level of seedling height reduction, EMS and NEUA gave less reduction of survival rate than other agents, and NMUA and NMUT likewise did less reduction of seed fertility.

At the same level of dosage given ($\text{mM} \times \text{time}$), the magnitude of inducing damage was in the order of $\text{NMUT} > \text{NMUA} \gg \text{EI} > \text{NEUA} \gg \text{EMS}$ for survival rate, $\text{NMUT} > \text{NMUA} \gg \text{NEUA} > \text{EI} \gg \text{EMS}$ for seedling height and $\text{NEUA} \geq \text{NMUA} \gg \text{NMUT} \geq \text{EI} \gg \text{EMS}$ for seed fertility. Thus, methylating agents (NMUA and NMUT) were physiologically more harmful than ethylating ones (EI, EMS and NEUA).

(2) Mutations in M_2 generation

Chlorophyll mutations

As clearly seen in Table 1, the frequency of chlorophyll mutants among M_2 seedlings increased markedly with increasing dosage in all the agents except NMUT. Within the dose ranges examined in the M_2 generation, the highest frequency through overall treatments was 8.90% in the treatment with 34 mM NEUA. When the highest frequency of each treatment is picked up, NEUA is followed by EMS, EI, NMUA and NMUT in descending order. This indicates that ethylating agents induce chlorophyll mutations at higher rate than methylating agents.

Table 2 indicates the spectrum of induced chlorophyll mutations. The frequencies were calculated from pooled data in each agent, since different doses of an agent produced almost identical spectra. Chlorophyll-deficient types other than *albina*, *viridis*, *xantha*

Fig. 1. Biological effects of alkylating agents in M_1 generation. Hour in parenthesis shows the duration of treatment. Presoaking and post-washing were done for 4 hrs. and 18 hrs., respectively, through all the chemicals.

Table 1. Frequency of chlorophyll mutations in M_2 generation

Agent	Mutagenic treatment		No. of tested M_2 seedlings	Mutation frequency* (%)
	Dosage mM	hr.		
Control	—	—	18,645	0
EI	20	— 3	20,355	0.45
	40	— 3	18,570	1.42
	60	— 3	15,760	2.85
	80	— 3	12,781	4.06
EMS	50	— 7	15,239	0.08
	80	— 7	18,232	1.60
	120	— 7	11,903	3.34
	150	— 7	10,853	4.95
NEUA	8	— 5	16,722	2.27
	17	— 5	10,960	4.89
	25	— 5	8,502	6.47
	34	— 5	7,154	8.90
NMUA	2	— 5	19,097	0.21
	5	— 5	16,356	0.20
	10	— 5	14,515	1.43
	20	— 5	5,295	1.59
NMUT	4	— 5	18,753	0.22
	7	— 5	21,145	0.35
	14	— 5	18,522	0.52
	21	— 5	11,698	0.01

* Mutants per 100 M_2 seedlings.

and *striata* were induced in very low frequencies, so they were pooled as "others". As can be seen from Table 2, a distinct difference of the spectrum was observed between NMUT and other four agents. NMUT produced exclusively *viridis* and *striata*. Differences were found also among the four agents though they were not so large: EI and EMS produced *xantha* and *others* respectively in significantly lower proportions than the other agents, while NEUA and NMUA produced *others* and *striata* respectively in significantly higher proportion.

Mutations in agronomic characters

As shown in Table 3, the mean values of heading date did not significantly differ from the control population in all the treatments except 25 mM NEUA, while those of culm length were significantly reduced than the control in all the treatments except those of NMUT. The variances of both characters significantly increased as compared with those of the control in all the treatments except those of NMUT, and the increase became greater with increasing dosage of the agents other than NMUT. Variances in the treatments with NMUT decreased with increasing dosage. The data suggest that NMUT has not much potentiality of inducing the mutations in agronomic characters. The data also indicate that the agents other than NMUT induce mutations in both directions of early and late heading, while they induce mutations mainly in one direction of short culm.

The plants which headed earlier or later by at least 4 days than the mean of the control population were regarded as variants in heading date according to the method as mentioned before. All over the treatments, there were observed variants with 22

Table 2. Spectrum of chlorophyll mutation

Agent	Frequency of mutants in percent					Total no. mutants classified
	<i>albina</i>	<i>viridis</i>	<i>xantha</i>	<i>striata</i>	<i>others</i> *	
EI	38.7	50.0	2.7**	4.2	4.4	1,323
EMS	39.7	43.5	8.0	6.7	2.1**	1,238
NEUA	41.2	35.6	9.1	5.6	8.4**	2,108
NMUA	37.6	34.9	9.6	14.3**	3.6	364
NMUT		88.6**		11.4**		211
All agents	38.3	43.2	6.9	6.3	5.2	5,244

others: *albo-viridis*, *virido-albina*, etc.

** Significant at 1% level.

Table 3. Mutagenic effects on heading date, culm length and seed fertility in M_2 population

Agent	Treatment Dosage mM—hr.	No. of tested		Heading date			Culm length			Freq. of sterile variants ²⁾ (%)
		M_1 pani- cles	M_2 plants	Mean ¹⁾ (days)	Vari- ance (days)	Freq. of variants ²⁾ (%)	Mean (cm)	Variance (cm)	Freq. of variants ²⁾ (%)	
Control	—	95	1,427	33.24	1.53	0.21	75.12	23.34	0.91	0.00
EI	40—3	100	1,515	33.33	3.66**	6.27	74.05	59.96**	6.87	7.19
	60—3	100	1,488	33.40	5.61**	9.34	73.87	89.99**	10.62	12.50
	80—3	100	1,374	33.27	8.50**	13.32	71.07**	108.48**	17.76	20.38
EMS	80—7	100	1,428	33.18	3.44**	5.25	73.25**	64.36**	7.28	7.71
	120—7	100	1,431	33.06	5.97**	9.64	71.80**	90.15**	13.28	15.93
	150—7	100	1,430	33.44	7.95**	12.52	68.61**	92.50**	19.65	21.27
NEUA	8—5	100	1,529	33.15	3.82**	5.95	73.16**	49.70**	7.33	5.62
	17—5	100	1,472	33.68	7.85**	15.01	68.57**	92.53**	20.79	19.63
	25—5	99	1,364	34.39**	9.75**	19.72	68.85**	115.21**	25.00	43.91
NMUA	5—5	100	1,546	33.41	2.77**	2.91	74.21	40.15**	3.88	7.50
	10—5	100	1,511	33.00	3.32**	5.23	73.33**	42.38**	4.83	7.54
	20—5	68	974	33.09	6.07**	11.30	71.36**	65.99**	11.70	11.26
NMUT	4—5	100	1,394	33.43	2.11	0.14	76.00	36.71*	1.22	1.87
	7—5	100	1,562	33.43	1.90	0.64	74.32	30.71	3.39	3.39
	14—5	100	1,569	33.41	1.32	0.13	75.22	20.06	1.28	2.10

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2) Variants per 100 M_2 plants. Variants: Plants outside the confidence limits ($=0.01$). (See text)

* and **: Significant at 5 and 1% levels, respectively.

days earlier to 13 days later heading in comparison with the mean of original variety. The variants in culm length were likewise defined as the plants whose culm lengths were outside the control limits, 64 and 85 cm. Their upper- and lowermost culm length were 38 cm and 110 cm, respectively. The frequency of the variants may not be consistent with that of mutants induced, but it can be highly significant index for the estimation of mutagenic effect of alkylating agents as inferred also from the previous report (Ko *et al.* 1969).

In all the agents except NMUT, frequencies of the variants in heading date, culm length and seed fertility increased with increasing dosage. Through the three agronomic characters, ethylating agents induced variants at higher rate than methylating ones alike the case of chlorophyll mutations: The highest frequency was obtained in the treatment with 25 mM of NEUA.

Chlorophyll mutation appearing on M_2 seedlings has widely been used for testing the mutagenicity of mutagen. As seen in Fig. 2, the present experiment indicated that the frequency of variants in each of the two agronomic characters, heading date and culm length, was quite exactly proportional to that of

Fig. 2. Relationship between the frequencies of variants in two agronomic characters and the frequency of chlorophyll mutants.

chlorophyll mutations in all the agents applied, with the exception of one treatment (NMUA 20 mM). The linear relationship observed here suggests that the frequency of chlorophyll mutations can be practically adopted as an index for mutagenicity of an alkylating agent.

(3) Mutagenic effectiveness and efficiency

The mutagenic effectiveness and efficiency of the agents used are given in Table 4. After the method of KONZAK *et al.* (1965), The effectiveness was calculated from M/CT, and the efficiency was done from M/I, M/S and M/SM, where M represents the frequency of mutants or variants, C presents concentration in millimoles, T does time of treatment in hours, I expresses injury (% reduction in seedling height), S means sterility (% reduction in M_1 seed fertility), and SM is frequency of sterile variants.

As indicated in Table 4, the effectiveness differed markedly among the agents. NEUA was most effective through the three observed characters, followed by NMUA, EI, NMUT and EMS in turn; About 15-fold dosage was required for EMS as compared with NEUA to obtain an equal frequency of mutants or variants.

The data also revealed differences of the efficiency among the agents used, but these differences varied with the different bases of calculation. In the efficiency on the basis of M_1 injury (M/I), the order of agents was NEUA $\gg$ EI $>$ EMS $\gg$ NMUA $>$ NMUT: Methylating agents were less efficient than ethylating agents. Almost the same order was obtained also on the basis of M_1 sterility (M/S), though the differences were small especially among ethylating agents.

Regarding the efficiency on the basis of sterile variants frequency (M/SM), distinct differences were not observed among the agents except for NMUT. NMUT showed less efficiency particularly in induction of the variants in heading date. In heading date, M/SM was affected by the dosage of each agent; the efficiencies of EI, EMS and

Table 4. Mutagenic effectiveness and efficiency of different alkylating agents

Treatment		Chlorophyll				Heading date				Culm length			
Agent	Dosage mM—hr.	Effec- tiveness M/CT	Efficiency			Effec- tiveness M/CT	Efficiency			Effec- tiveness M/CT	Efficiency		
			M/I	M/S	M/ SM		M/I	M/S	M/ SM		M/I	M/S	M/ SM
EI	40—3	1.2	0.07	0.10	0.20	5.2	0.30	0.45	0.82	5.7	0.33	0.49	0.96
	60—3	1.6	0.10	0.10	0.23	5.2	0.32	0.33	0.75	5.9	0.37	0.37	0.85
	80—3	1.7	0.07	0.10	0.20	5.6	0.24	0.33	0.65	7.4	0.33	0.43	0.87
EMS	80—7	0.3	0.06	0.13	0.21	0.9	0.18	0.42	0.68	1.3	0.25	0.58	0.94
	120—7	0.4	0.08	0.10	0.21	1.2	0.23	0.28	0.61	1.6	0.31	0.39	0.83
	150—7	0.5	0.08	0.12	0.23	1.2	0.20	0.29	0.59	1.9	0.31	0.46	0.92
NEUA	8—5	5.7	0.16	0.15	0.40	14.9	0.42	0.39	1.06	18.3	0.52	0.48	1.30
	17—5	5.8	0.20	0.13	0.25	17.7	0.60	0.41	0.77	24.4	0.84	0.57	1.06
	25—5	5.2	0.13	0.13	0.15	15.8	0.38	0.38	0.45	20.0	0.49	0.48	0.57
NMUA	5—5	0.8	0.01	0.02	0.03	11.6	0.10	0.33	0.38	15.5	0.14	0.45	0.52
	10—5	2.9	0.03	0.07	0.19	10.5	0.10	0.27	0.69	9.7	0.10	0.25	0.64
	20—5	1.6	0.02	0.05	0.14	11.3	0.14	0.32	1.00	11.7	0.15	0.34	1.04
NMUT	4—5	1.1	0.01	0.03	0.12	0.7	0.01	0.02	0.08	6.1	0.08	0.18	0.65
	7—5	1.0	0.01	0.04	0.10	1.8	0.02	0.08	0.19	6.7	0.08	0.41	1.00
	21—5	0.7	0.01	0.06	0.25	0.2	0.01	0.02	0.06	1.8	0.02	0.15	0.61

M/I, M/S, M/SM : On the basis of M_1 seedling injury, M_1 seed fertility and M_2 sterile-variants frequency, respectively (See text).

CT : Dosage (concentration $\times$ time).

NEUA decreased as dose increased, while a reverse trend was observed with NMUA.

Discussion

Comparative studies of different kinds of alkylating agents have revealed various differences in the degree of physiological damages and mutagenic effects (BAUTZ and FREESE 1960, ARNASON *et al.* 1962, KONZAK *et al.* 1965). The present study also suggested that the potencies based on dosage (mM $\times$ time) of the agents used was in the order of NMUT>NMUA»NEUA>EI»EMS for the reduction of M_1 seedling height, and NEUA»NMUA»EI>NMUT>EMS for the induction of mutations.

According to previous studies on the effect of alkyl alkanesulfonates, methylating agent is more toxic and more mutagenic than its corresponding ethylating agent on equimolar basis (MINOCHA and ARNASON 1962, ROSS 1962, EHRENBERG *et al.* 1966). DESAI *et al.* (1975) treated durum wheat with NMUA and NEUA at an equimolar dose and reported that NMUA was more toxic to plant survival as well as to spike fertility and more frequently induced both chlorophyll and viable mutations than NEUA. VELEMÍNSKÝ *et al.* (1970) also indicated in barley and arabidopsis that NMUA required less mM concentration than NEUA for inducing not only the same degree of seedling injuries but also the same frequency of mutations. In the present investigation, however, NMUA was more toxic but less mutagenic than NEUA at an equimolar dose. For elucidation of this difference between the above reports and the present study, characteristics of experimental organism and various other factors that influence the biological response, such as the molarity, pH and temperature of chemical solution, must also be taken into consideration as pointed out by KONZAK *et al.* (1965) and EHRENBERG (1971).

It is generally known that ethylating agents are less toxic and therefore more efficient as mutagen than methylating agents (RAO and NATARAJAN 1965, EHRENBERG 1971, KAMRA and BRUNNER 1977). The present experimental results are not exceptions either. Namely, judging from the mutagenic efficiencies based on the seedling injury and sterility, NEUA was most efficient through the three kinds of mutations, followed by EI, EMS, NMUA and NMUT in descending order (Table 4). However, DESAI *et al.* (1975) reported in durum wheat that the efficiency of NEUA was much lower than that of NMUA. RAMUL (1970) also showed in sorghum that NEUA was less efficient than EMS. Such a discrepancy in relative mutagenic efficiency between authors may also be due to the differences of experimental organism and treatment condition.

It is interesting that the difference among agents was smaller in M/S than in M/I. This suggests that sterility is more closely related to mutation frequency than injury, in other words, the greater part of sterility does not result from physiological damages but from genetic causes as stood for by embryo-lethal mutation (SATO and GAUL 1967). M/S may thus nearly express the ratio of viable to embryonic-lethal mutations as indicated by KONZAK *et al.* (1965).

Low biological damage makes high-dose application possible. However, the treatment with high dose is not always desirable in mutation breeding, because the mutants induced with higher doses are often accompanied by undesired traits such as sterility and reduced vitality. MESKEN and VAN DER VEEN (1968) suggested that the relative efficiency of

different mutagens had better be estimated on the basis of the sterility in M_2 than in M_1 . Distinct differences in M/SM were not recognized among the agents used in the present study, with the exception of less efficient for heading date in the treatment with NMUT. But a reduction trend of M/SM at increased doses was recognized in the three ethylating agents, especially in NEUA. This means that in ethylating agents the dose-dependent increase of sterile mutants is more rapid than that of mutants in heading date and culm length.

NMUT proved here to be least effective and least efficient among the mutagens used. However, NMUT was really unique and interesting in that it exclusively induced *viridis* and *striata* types of mutation. As to the treatment with NMUT, TANO and YAMAGUCHI (1969) found in barley that only *viridis*-type mutation appeared after the treatment, while SATO and KAWAI (1970) observed in rice not only *viridis* but also *albina*, *xantha* and *others*. It is not yet unknown from what mechanism this uniqueness of NMUT originated.

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人為突然変異の利用に関する育種学的研究 XIII. 水稻における 数種アルキル化物質の突然変異誘起効果

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高等植物において突然変異原として広く用いられている5種類のアルキル化物質, ethylenimine (EI), ethyl methanesulfonate (EMS), N-nitroso-N-ethyl urea (NEUA), N-nitroso-N-methyl urea (NMUA) および N-nitroso-N-methyl urethane (NMUT) の実用形質に関する突然変異誘起効果を比較するため, 水稻品種銀坊主の気乾種子をあらかじめ4時間浸漬したのち, これら物質の種々の濃度の溶液で浸漬処理を行い, 突然変異誘起力 (mutagenic effectiveness: 単位処理量当りの突然変異出現率) および突然変異誘起効率 (mutagenic efficiency: 単位障害当りの突然変異出現率) を調べた。

処理当代の幼苗生存率, 幼苗草丈および種子稔性に対する処理効果は, 各突然変異原とも処理濃度の増加に伴って増大したが, これら3形質の濃度反応様式はそれぞれ突然変異原の間で明らかに異なった。幼苗草丈の低下が同程度の処理間で比較したところ, 処理効果は, EIの場合は3形質いずれについても同程度であったが, EMS および NEUA の場合は幼苗生存率, NMUA および NMUT の場合は種子稔性で相対的に低かった。

処理次式における葉緑, 出穂日, 稈長および種子稔性に関する変異体出現率は, NMUT を除き処理量の増加とともに高くなったが, どの形質についても, 最大値でみると NEUA が最も高く, ついで EMS, EI, NMUA, NMUT の順となった。突然変異誘起力では, 葉緑, 出穂日, 稈長いずれにおいても NEUA > NMUA > EI > NMUT > EMS の順となり, NEUA と EMS の間には約 15 倍の差があった。また突然変異誘起効率では, 3形質とも, 処理当代に生じる障害を基準とした場合は NEUA > EI > EMS > NMUA > NMUT の順となり, エチル化物質はメチル化物質に比べ高かったが, 処理次代に出現する遺伝的障害 (種子不稔変異) を基準とした場合は突然変異原間に明らかな差異が認められなかった。

以上の結果より, 供試アルキル化物質間には突然変異誘起効果に著しい差異があり, エチル化物質はメチル化物質に比べて実用形質に関する突然変異を誘起しやすいこと, とりわけ NEUA は突然変異誘起力に優れるのみならず処理当代に与える障害が最も少ない有効な突然変異原であることが明らかとなった。しかしながら同時に, エチル化物質では, 処理量の増加に伴う種子不稔変異の増大がメチル化物質に比べて相対的に大きい傾向が認められた。このことは, 突然変異誘起効果の大きい突然変異原を作物育種の場に用いる際には, 最適処理量の決定に当って種子不稔変異などの遺伝的障害の出現率に対する配慮が必要であることを示唆している。

なお本実験で用いたいずれの突然変異原においても, 出穂日および稈長に関する変異体の出現率と葉緑突然変異体出現率との間に一定の比例関係が認められた。このことは葉緑突然変異出現率が各種アルキル化物質の育種的有用性の早期判定指標として有効であることを示している。