Res. Bull. Univ. Farm Hokkaido Univ. 26 : 31~36 (1989)

Comparison of Mutagenic Effectiveness by the Treatment of Fertilized Egg Cells with *N*-methyl-*N*-nitrosourea and Ethylmethanesulfonate in Rice (*Oryza sativa* L.)

Masahiko MAEKAWA, Toshihiko MAEKAWA*, Masao HIWATARI, Takahiro TSUNODA and Fumiji KITA

(Experiment Farms, Faculty of Agriculture, Hokkaido University, Sapporo, 060 Japan)

(* Plant Genetic Resources Center of Hokkaido, Takikawa 073, Japan)

(Received October 31, 1988)

Introduction

It is clearly shown that mutations induced by gamma-rays irradiation or chemical mutagens have been very useful for crop improvement[3,4]. However, in order to utilize artificially-induced mutations as a more effective tool for breeding, it is required to increase mutation frequency and to control the mutation spectrum[8]. Recently, to increase the mutation frequency, SATOH and OMURA[6] and ZHOU *et al.*[10] applied fertilized egg cells treatment with alkylating agents in rice and wheat, respectively, while GAUL[2] treated the zygotes of barley by gamma-rays. They reported that this treatment was more effective than dry seeds treatment, because at one cell stage the treatment was conducted and no chimeric sectors derived from mutated cell were formed. As the alkylating agents, SATOH and OMURA[5,6] used *N*-methyl-*N*-nitrosourea (MNU) and SATOH and OMURA[5,8] pointed out that ethyl methanesulfonate (EMS) treatment of zygote in rice was less effective than MNU treatment. On the other hand, ZHOU *et al.*[10] applied EMS and obtained high mutation frequency in wheat.

In this study, mutagenic effectiveness by the treatment of fertilized egg cells with MNU and EMS was compared in Japonica and Indica rice varieties.

Materials and Methods

Rice varieties used in this experiment were Shiokari, Yukihikari (Japonica type), E-48 (Rokujunichi-ouso, Indica type) and 84ACC741 (Linkage marker). The concentration of MNU was 1.5 mM, adjusted to pH 4.8 according to SATOH and OMURA[6], and that of EMS was 24 mM with the method as described by ZHOU *et al.*[10]. This concentration of EMS was higher than that (10 and 15 mM) of SATOH and OMURA[5], in order to compare the mutagenic effectiveness by the treatment of fertilized egg cells with EMS and MNU. These varieties were grown until the flowering stage in the paddy fields of Agricultural Experiment Farm, Faculty of Agriculture, Hokkaido University in 1985, and were transferred to the green house and the spikelets which had already flowered on the

* Present address ; Kitami Agricultural Experiment Station of Hokkaido, Kun-neppu-cho, Tokoro-gun 079-14 Japan

day before the treatment, were cut off. Panicles which had spikelets flowered from 10 : 30 to 11 : 30 in the morning were dipped into MNU, EMS solutions and distilled water as control from 5 : 30 to 8 : 30 in the night. After the treatment, treated spikelets were washed in running tap water for 24 hours. In 1986, M_1 plants were grown and in 1987 mutated characters were examined in M_2 lines. Mutation frequency was expressed as number of M_2 lines which showed mutant characters/total of M_2 lines $\times$ 100.

Results and Discussion

Seed fertility of treated plants with MNU or EMS and the germination rate of M_1 plants were shown in Table 1. Shiokari and E-48 showed higher seed fertility by the treatment with MNU than that with EMS. On the other hand, Yukihikari and 84ACC741 showed higher seed fertility with EMS than that with MNU. Seed fertilities of Shiokari and 84ACC741 by MNU or EMS treatment were lower than those of control except for Yukihikari and E-48, while SATOH and OMURA[6] indicated that the treatment with 1.5 mM MNU for 3 hours did not reduce seed fertility. All four varieties showed lower germination rate of M_1 plants by the treatment with EMS than that with MNU. From this it was assumed that the treatment with EMS resulted in more immature seeds than that with MNU. Sterility of M_1 plants obtained by the treatment of fertilized egg cells with EMS and MNU was presented in Table 2. All four varieties showed a higher frequency of completely sterile M_1 plants by EMS treatment than that by MNU treatment. On the other hand, the percentage of normal fertile plants was markedly higher with MNU treatment than with EMS treatment. All MNU-treated M_1 plants of E-48 showed partial sterility. From these results, it was presumed that EMS was more harmful for seed fertility than MNU for fertilized egg cells treatment, although FUJIMOTO and YAMAGATA[1] indicated that methylating agents caused more damages than ethylating ones. In M_2 generation, as shown in Table 3, the frequency of chlorophyll mutation at seedling stage of Yukihikari and E-48 was increased by MNU treatment. On the other hand, that of Shiokari was decreased by MNU treatment and that of 84ACC741 by MNU treatment was similar with that by EMS treatment. As compared with the treatment of dry seeds of Yukihikari by gamma-ray irradiation, the treatment of fertilized egg cell with MNU and EMS showed a very high frequency of chlorophyll mutation at seedling stage of M_2 lines. Further, frequency of culm length mutation, including dwarf mutants mainly, of M_2 lines of each variety

Table 1. Seed fertility(S.F.) of treated plants with EMS and MNU and germination rate(G.R.) of M_1 plants obtained by the treatment of fertilized egg cells with EMS and MNU

	Treatment of fertilized egg cells with					
	EMS		MNU		Cont.	
Variety	S.F.	G.R.	S.F.	G.R.	S.F.	G.R.
Shiokari	59.4	69.1	69.5	90.5	79.0	97.1
Yukihikari	81.8	81.8	67.9	94.4	81.6	95.6
E-48*	74.9	54.9	86.8	71.9	84.2	91.4
84ACC741**	60.8	47.5	46.0	66.7	75.8	91.2

*; Rokujunichi-ousou(Indica type).
**; Linkage marker.

except for Shiokari by MNU treatment was very higher than that by EMS treatment. M_2 lines by MNU treatment of Shiokari showed a not so higher frequency of culm length mutation than that by EMS treatment and the frequency of culm length mutation of M_2 lines by MNU treatment of Shiokari was higher than half of that of M_2 lines of the other varieties. From these results, it was revealed that regarding induction of mutations in Indica rice as well as Japonica rice varieties, the treatment of fertilized egg cell with MNU was more effective than that with EMS, as SATOH and OMURA[5,8] indicated.

Table 2. Sterility of M_1 plants obtained by the treatment of fertilized egg cells with EMS and MNU

Variety	Mutagen	Sterility of M_1 plants: Complete sterility	Partial sterility	Normal	Total
Shiokari	EMS	8	22	1	31
		(25.8)	(71.0)	(3.2)	
	MNU	1	11	58	70
		(1.4)	(15.7)	(82.9)	
Yukihikari	EMS	21	30	22	73
		(28.8)	(41.1)	(30.1)	
	MNU	1	28	66	95
		(1.1)	(29.5)	(69.5)	
E-48	EMS	11	29	3	43
		(25.6)	(67.4)	(7.0)	
	MNU		61		61
			(100.0)		
84ACC741	EMS	8	3	8	19
		(42.1)	(15.8)	(42.1)	
	MNU		1	10	11
			(9.1)	(91.0)	
Yukihikari	20 kR*		1	1999	2000
			(0.1)	(100.0)	

*; Dry seeds treatment by gamma-ray irradiation.
The percentage was given in the parenthesis.

Table 3. Frequency of chlorophyll mutation and culm length mutation generated in M_2 lines obtained by the treatment of fertilized egg cells with EMS and MNU

Variety	Mutagen	No. of tested M2 lines	Chlorophyll mutation: No. of lines	Mutation frequency (%)	Culm length mutation: No. of lines	Mutation frequency (%)
Shiokari	EMS	23	3	13.0	5	21.7
	MNU	69	7	10.1	16	23.2
Yukihikari	EMS	52	7	13.5	14	26.9
	MNU	94	22	23.4	50	53.2
E-48	EMS	32	3	9.4	6	18.8
	MNU	61	16	26.2	27	44.3
84ACC741	EMS	11	2	18.2	2	18.2
	MNU	11	2	18.2	5	45.5
Yukihikari	20 kR*	2000	7	0.4	----	----

*; Dry seeds treatment by gamma-ray irradiation.

Although spikelets at 6 or 7 hours from pollination were treated with MNU or EMS in this study, ZHOU *et al.*[10] and SATOH *et al.*[9] described that treatment of fertilized egg cells at 15 or 14 hours after pollination with EMS or MNU, respectively, enhanced mutagenic effectiveness. Further, SATOH and OMURA[7,8] suggested from the study for induction of endosperm mutants that treatment of fertilized egg cells at various hours after pollination with MNU may make it possible to control mutation spectra. Consequently, treatment of fertilized egg cells with mutagens is a very valuable breeding-tool. When foreign rice genetic resources are utilized in rice breeding program, especially in Northern districts, in order to conquer the environmental limitation this treatment is a very efficient method. Further, for increasing the mutagenic effectiveness in the treatment of fertilized egg cells, to select a powerful mutagen is required. The results obtained in this study supported the result reported by SATOH and OMURA[5] that MNU is more efficient than EMS as a mutagen in the treatment of fertilized egg cells. According to SATOH and OMURA[8], *N*-ethyl-*N*-nitrosourea (ENU) gave rise to a similar effect to MNU in the treatment of fertilized egg cells, but *N*-methyl-*N*^{1}-nitro-*N*-nitrosoguanide (MNG) was ineffective.

Summary

This study was conducted to compare the mutagenic effectiveness by the treatment of fertilized egg cells with *N*-methyl-*N*-nitrosourea (MNU) and ethyl methanesulfonate (EMS) in Japonica rice varieties (Shiokari, Yukihikari and 84ACC741) and Indica rice variety (E-48). Spikelets of each variety at 6 or 7 hours after pollination were dipped into 1.5 mM MNU and 24 mM EMS solutions for 3 hours. Seed fertility of EMS-treated Shiokari and E-48 was lower than that of MNU-treated ones. On the other hand, seed fertility of MNU-treated Yukihikari and 84ACC741 was lower than that of EMS-treated ones. Three varieties except for Yukihikari showed lower seed fertility of treated plants than the control. Germination rates in M_1 populations from all EMS-treated varieties were lower than those in M_1 populations from all MNU-treated ones. A few completely sterile plants were observed in M_1 populations derived from EMS-treated plants of all varieties, while normally fertile plants increased in M_1 populations from MNU-treated plants of three varieties except for E-48. All M_1 plants from MNU-treated E-48 showed partial sterility. Chlorophyll mutation frequency increased in M_2 population derived from MNU-treated Yukihikari and E-48, but the differences of chlorophyll mutation frequency between M_2 populations from EMS- and MNU-treated Shiokari or 84ACC741 were not observed. Further, culm length mutation frequency in M_2 populations from MNU-treated Yukihikari, E-48 and 84ACC741 increased as much as twice of that in M_2 populations from EMS-treated ones. Culm length mutation frequency in M_2 population from MNU-treated Shiokari did not differ from that in M_2 population from EMS-treated one. Consequently, it was concluded that MNU was more powerful mutagen than EMS in the treatment of fertilized egg cells of Indica rice as well as Japonica rice varieties.

Acknowledgement

The authors wish to express their gratitude to Mr. Y. Ebe, Director of Plant Genetic Resources Center of Hokkaido, for his interest and encouragement.

References

1. FUJIMOTO, M. and YAMAGATA, H. : Studies on the utility of artificial mutations in plant breeding. XIII. Mutagenecity of several alkylating agents in rice. *Jpn. J. Breed.* **32** : 17-25. 1982
2. GAUL, H. : Mutation in plant breeding. *Radiation Bot.* **4** : 215-232. 1964
3. KAWAI, T. : Radiation breeding -25 years and further on. *Gamma Field Symposia* **25** : 1-36. 1986
4. KONZAK, C. F., KLEINHOFS, A. and ULLRICH, S. E. : Induced mutations in seed-propagated crops. *in* Plant breeding reviews. Janick, J. edited. **2** : 13-72. 1984
5. SATOH, H. and OMURA, T. : Effects of chemical mutagens applied on early stages of embryonic development in rice. II. Effects of EI and EMS. *Jpn. J. Breed.* **25** (*Suppl.* 2). 36-37. 1975 (in Japanese)
6. SATOH, H. and OMURA, T. : Induction of mutation by the treatment of fertilized egg cell with *N*-methyl-*N*-nitrosourea in rice. *J. Fac. Agr., Kyushu Univ.* **24** : 165-174. 1979
7. SATOH, H. and OMURA, T. : New endosperm mutations induced by chemical mutagens in rice, *Oryza sativa* L.. *Jpn. J. Breed.* **31** : 316-326. 1981
8. SATOH, H. and OMURA, T. : Mutagenesis in rice by treating fertilized egg cells with nitroso compounds. *in* Rice genetics. Proceedings of the International Rice Genetics Symposium. 707-717. 1986
9. SATOH, H., SHIRAISHI, M., CHAEN, K., MORIMOTO, T. and OMURA, T. : Induction of mutation by the treatment of fertilized egg cells with MNU in rice variety "Taichung 65". *Jpn. J. Breed.* **37** (*Suppl.* 2) : 338-339. 1987 (in Japanese)
10. ZHOU, Z., GU, A., WANG, Q. and GENG, Y. : Effects of chemical mutagenesis on treating zygotes in spring wheat. *Acta Genet. Sinica* **7** : 341-346. 1980 (in Chinese with English summary)

北海道大学農学部農場研究報告　第26号：31－36（1989）

イネのN-メチル-N-ニトロソ尿素とエチルメタンスルフォネートによる受精卵処理の突然変異誘起効果の比較

前川　雅彦・前川　利彦*・飛渡　正夫
角田　貴敬・喜多富美治
（北海道大学農学部附属農場）
（*道立遺伝資源センター
現在：道立北見農業試験場）

（1989年10月31日受理）

摘　　要

イネの受精卵の1細胞期に突然変異源を処理すると高い突然変異率を生ずることが知られている。突然変異源としてイネではN-メチル-N-ニトロソ尿素(MNU)，小麦ではエチルメタンスルフォネート(EMS)で効果が高いと報告されている。従来は，イネではEMSについてはMNUより効果が高いとされているが，本研究ではイネの受精卵処理におけるMNUとEMSの突然変異誘起効果を日本型とインド型イネについて比較検討した。供試材料は，「しおかり」，「ゆきひかり」（日本型），E-48（インド型）及び84ACC741（標識遺伝子型系統）で，開花後6～7時間後の穎花をMNU 1.5 mM，EMS 24 mM及び水（対照）に浸漬処理した。処理当代の種子稔性は，EMS処理で低下する場合（「しおかり」，E-48），逆にMNU処理で低下する場合（「ゆきひかり」，84ACC741）とがあった。また，いずれも「ゆきひかり」を除いては無処理区より低下していた。M_1世代の発芽率は，いずれもEMS処理で低下していた。次に，M_1世代の不稔性を調べてみると，EMS処理でいずれの場合も，完全不稔個体を多く析出していた。一方，MNU処理では，E-48を除いていずれも正常稔性を示す個体が多かった。E-48のMNU処理では全個体が半不稔性を示していた。M_2世代における葉緑素突然変異頻度については，「ゆきひかり」・E-48のMNU処理で効果が高く，「しおかり」及び84ACC741のMNUとEMS処理では同程度の効果であった。一方，稈長に関する突然変異頻度については「しおかり」のMNUとEMS処理で同程度の効果があったが，他はいずれもMNU処理の効果が高かった。以上のことから，日本型ばかりでなくインド型イネでも受精卵処理においては，MNUがEMSより高い突然変異誘起効果を生ずると結論された。