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CHEMICAL MUTAGENESIS IN MANHAR VARIETY OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Treatments with mono- and bifunctional ethylated (NEU, NDEU) and methylated (NMU, NDMU) urea and ethyl methane sulphonate (EMS) in Manhar, an early maturing (120 days) variety of *indica* rice reduced the germination, seedling survival, pollen fertility and spikelet fertility percentage in M_1 generation. NEU produced the highest frequency of viable mutations in the M_2 generation, on the basis of segregating M_2 progenies as well as mutants/100 M_2 seedlings. Treatments with NEU (6 h) also produced the widest spectrum and maximum relative frequency of viable mutations in M_2 generation. The relative mutation frequency of monofunctional compounds NEU and NMU was larger than their respective bifunctional compounds NDEU and NDMU. The most effective and efficient mutagens were 0.01% NMU and 0.05% NDEU, respectively.

Key words: Mutagen, mutations, effectiveness, efficiency, rice.

Mutation breeding has been widely used for the improvement of various plant characters from time to time. Experiences have shown that the frequency of desirable mutations depends upon the effectiveness and efficiency of the mutagens used [1, 2]. The monofunctional ethylated and methylated urea have been found to be more effective and efficient than the alkylating agents and physical mutagens in crops, viz. barley [3, 4], lentil [5, 6] and rice [7, 8]. However, their effectivity and efficiency have not been compared with the respective bifunctional compounds. The present study deals with the frequency and spectrum of induced viable mutations and the results on the effectiveness and efficiency of mono and bifunctional ethylated and methylated urea in an early maturing, semidwarf *indica* rice variety Manhar.

MATERIALS AND METHODS

Husked rice seeds presoaked in distilled water for 4 h were treated at 20°C under controlled conditions with freshly prepared 0.03%, 0.04%, 0.05%, 0.06% and 0.07% aqueous

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solutions of N-nitroso-N-ethyl urea (NEU) and N-nitroso-N-diethyl urea (NDEU); 0.01%, 0.02%; 0.03%, 0.04% and 0.05% N-nitroso-N-methyl urea (NMU) and N-nitroso-N-dimethyl urea (NDMU); 0.25%; 0.50%, 0.75%, 1.0% and 1.25% ethyl methane sulphonate (EMS) for 6 and 12 h. The treated seeds were thoroughly washed in running tap water for one hour and sown in Petri dishes for germination. One week old seedlings were transferred to pots, and 25 days later the seedlings were transplated in the main field at 10 x 20 cm spacing.

The biological effects of different treatments in the M_1 generation were evaluated with respect to germination, seedling survival, pollen fertility and spikelet fertility percentage. The M_2 generation was grown as panicle progenies.

The mutation frequency was calculated following Gaul [9] and the mutagenic effectiveness and efficiency of different treatments were worked out following Konzak et al. [1].

RESULTS AND DISCUSSION

M_1 GENERATION

All the mutagens reduced the germination of treated seeds and seedling survival in the M_1 generation, for which EMS was lethal at 1.25% concentration. The mean pollen and spikelet fertility were also reduced in all the treatments. NEU resulted in the maximum reduction of mean pollen and spikelet fertility. However, when various concentrations of

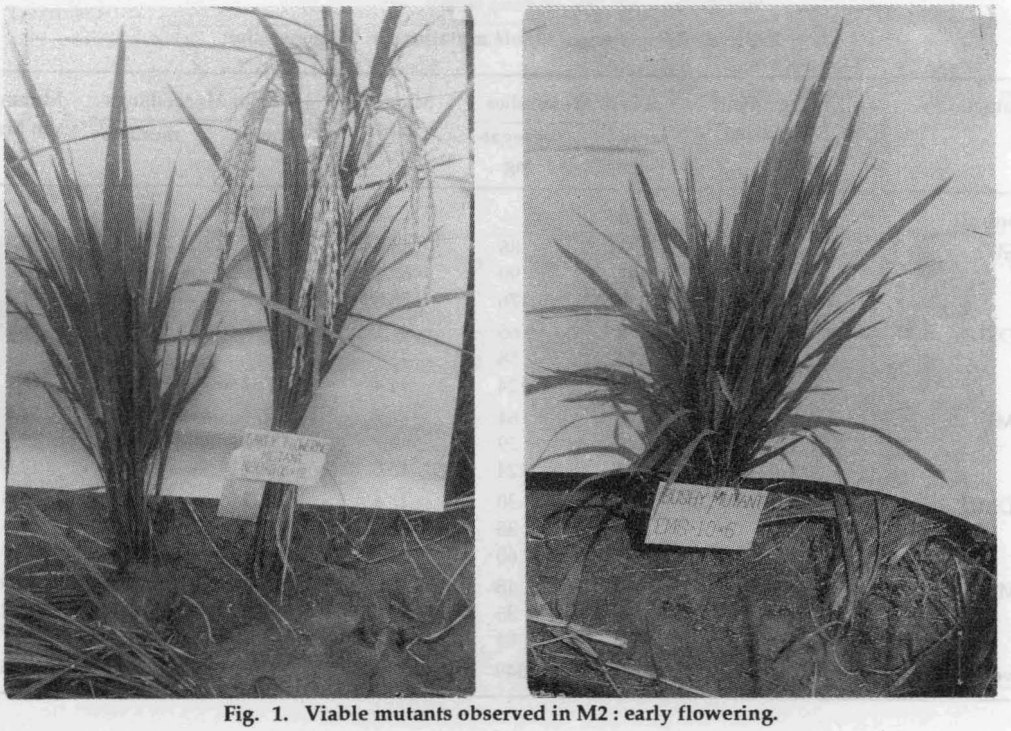

Fig. 1. Viable mutants observed in M2 : early flowering.

different chemicals were compared, it was found that treatments with 0.03% NMU (6 h) and NDMU (12 h) produced more than 50% sterile pollen. The effects of NDMU were more severe at 12 h duration for all the characters than 6 h.

Failure of homologous pairing during meiosis could be the main cause of high pollen sterility. Sterility may also be caused due to the physiological damage produced by the hydrolytic products of the alkylating chemicals. Gaul [10] has suggested that the chromosomal aberrations are probably the major effect of all mutagenically induced pollen sterility. The actual reason of sterility caused by these chemical mutagens may be gene mutation or, more probably, "invisible deficiencies," the frequency of which may be more than that induced by irradiation.

Though partial pollen sterility should not affect the spikelet fertility in rice as there is abundance of microspores during fertilization, the damage to the nuclei in female gametes can not be ruled out. Degeneration of the egg nucleus in the embryo sac and embryo abortion leading to spikelet sterility in M_1 generation has been observed in rice [11].

FREQUENCY OF VIABLE MUTATIONS IN M_2 GENERATION

The frequency of viable mutants was recorded in terms of segregating families and mutant seedlings in the M_2 population (Table 1). A perusal of the table revealed that the mutation frequency, on the basis of segregating M_2 progenies (%) as well as mutants/100

Table 1. Frequency of viable mutations in M_2 generation

Mutagen	Treatment duration	No of M_1 families		Mutants in M_1 (%)	No. of M_2 seedlings		Mutants in M_2 (%)
		scored	segregating		scored	mutants	
Control		30	—	—	792	—	—
NEU	6 h	177	86	48.6	3641	561	15.4
	12 h	133	90	67.7	2521	514	20.4
	Total	310	176	56.8	6162	1075	17.4
NDEU	6 h	140	66	47.1	2878	183	6.3
	12 h	139	58	41.7	2874	234	8.1
	Total	279	124	44.4	5752	417	7.2
NMU	6 h	150	64	42.7	2771	402	14.5
	12 h	145	59	40.7	2680	286	10.7
	Total	295	124	42.0	5451	688	12.6
NDMU	6 h	161	30	18.6	3120	109	3.5
	12 h	144	35	24.3	2815	235	8.3
	Total	305	65	21.3	5935	344	5.7
EMS	6 h	113	48	42.5	2513	260	10.3
	12 h	109	36	33.0	2474	178	7.2
	Total	222	84	37.8	4987	438	8.7
Over all treatments		1442	549	38.1	28287	2962	10.5

M_2 seedlings was highest in NEU. The 12 h treatment produced higher frequency of viable mutations than 6 h treatment. The mutant percentage in the population of bifunctional compounds, NDEU and NDMU was markedly lower than the respective monofunctional compounds, NEU and NMU.

SPECTRUM AND RELATIVE FREQUENCY OF VIABLE MUTATIONS

The spectrum and relative frequency of viable mutations varied with the mutagen and the duration of the treatment (Table 2). Data over all the treatments showed that the partially sterile mutants were most frequent. These were followed by dwarfs, highly sterile, late flowering, medium tall height and medium slender grains, early flowering, profuse tillering, narrow and dark green leaved, semispreading and bushy types. A variety of viable mutations affecting various morphological characters have been reported in rice earlier also [12–14].

Perusal of data for different treatments (Table 2) indicated that the widest spectrum and highest relative frequency of viable mutations occured in NEU. The relative mutation frequency of monofunctional nitroso compounds, NEU and NMU was higher than their

Table 2. Spectrum and relative frequency of viable mutations in M_2 generation

Treatment		Viable mutants and their percentage												
		tall	dwarf	partially sterile	highly sterile	early	late	medium slender grained	profuse tillering	medium tall and medium plants slender grains	semi-spreading	bushy	narrow dark green leaved	over all mutant types
NEU	6 h	4.28	10.52	52.41	6.77	0.18	5.88	4.27	1.78	12.12	—	—	—	18.93
	12 h	7.39	12.45	63.62	9.14	0.58	2.33	—	2.52	—	—	—	—	17.35
NDEU	6 h	—	27.32	55.19	4.92	7.10	4.98	—	—	—	—	—	—	6.18
	12 h	—	18.80	54.27	1.28	11.54	2.61	7.69	3.84	—	—	—	—	7.90
NMU	6 h	0.50	9.95	58.20	4.47	5.22	5.20	11.94	—	4.73	—	—	—	13.57
	12 h	1.02	6.99	80.42	10.13	—	1.39	—	—	—	—	—	—	9.65
NDMU	6 h	—	3.66	81.65	2.75	—	11.01	—	—	—	—	—	—	3.68
	12 h	0.85	3.82	43.40	0.43	—	18.29	26.38	1.70	—	5.53	—	—	7.93
EMS	6 h	—	8.08	72.69	5.00	—	0.76	—	—	—	—	4.23	9.23	8.78
	12 h	—	11.23	57.86	3.37	—	12.92	—	—	14.61	—	—	—	6.00
Over all treatments		2.32	11.17	60.97	5.97	2.19	5.57	5.13	1.21	3.81	0.44	0.37	0.81	—

respective bifunctional compounds, NDEU and NDMU. NEU [7] and NMU [15] have been reported to produce a high frequency of viable mutations in rice.

The monofunctional compounds gave a wider spectrum and higher frequency of viable mutations at 6 h treatment, while the spectrum and relative frequency of viable mutations was higher for 12 h treatment in case of bifunctional compounds. EMS also produced a wider spectrum and higher frequency of mutations at 6 h treatment.

Table 3. Mutagenic effectiveness and efficiency in M_2 generation

Treatment (T)	Spikelets sterility percentage (S)	Mutant spikes percentage (Msp)	Mutagenic effectiveness (Msp/T)	Mutagenic efficiency (Msp/S)
NEU 0.03%	25.2	46.3	183.5	1.8
NEU 0.04%	44.0	75.3	231.2	1.7
NEU 0.05%	56.9	56.8	142.3	1.0
NEU 0.06%	45.9	62.1	123.3	1.3
NEU 0.07%	60.6	81.9	142.2	1.3
Mean	—	—	164.5	1.4
NDEU 0.03%	22.7	40.3	179.0	1.8
NDEU 0.04%	23.0	44.7	141.9	1.9
NDEU 0.05%	14.5	47.0	118.0	3.2
NDEU 0.06%	32.3	48.9	98.0	1.5
NDEU 0.07%	19.8	43.3	81.3	2.2
Mean	—	—	123.6	2.1
NMU 0.01%	31.4	32.8	426.7	1.0
NMU 0.02%	34.1	40.0	247.5	1.2
NMU 0.03%	44.2	40.0	180.5	0.9
NMU 0.04%	33.7	49.7	143.2	1.5
NMU 0.05%	35.1	46.3	123.3	1.3
Mean	—	—	224.3	1.2
NDMU 0.01%	18.8	21.4	236.7	1.1
NDMU 0.02%	28.4	35.0	187.5	1.2
NDMU 0.03%	31.9	19.1	79.4	0.6
NDMU 0.04%	22.0	21.6	69.4	1.0
NDMU 0.05%	20.6	14.1	38.6	0.7
Mean	—	—	122.3	0.9
EMS 0.25%	27.8	35.8	18.1	1.3
EMS 0.50%	32.1	29.3	7.4	0.9
EMS 0.75%	42.0	40.0	5.3	0.9
EMS 1.00%	37.5	42.5	6.0	1.3
Mean	—	—	9.2	1.1

EFFECTIVENESS AND EFFICIENCY OF MUTAGENS

The effectiveness and efficiency of different treatments (Table 3) indicated that NMU was the most effective mutagens for the production of viable mutations. It was followed by NEU, NDEU, NDMU and EMS. N-nitroso-N-ethyl urea (NEU) has been reported to be similar to NMU in inducing mutation in fertilized egg cells of *japonica* rice. Both these chemicals were found to be superior to N-methyl-N-nitro-N-nitrosoguanide (MNG) in mutagenicity [8]. NEU has been reported to more effective than EI and gamma-rays in lentil also [6].

NDEU appeared to be the most efficient mutagen, followed by NEU, NMU, EMS and NDMU. Rao [16] reported that 0.03% NMU and 0.01% MMS were most efficient chemical mutagens for Tella Hamsa and IR 24 varieties of rice. On the other hand, Siddiq and Swaminathan [17] found that EMS was most efficient mutagen followed by gamma-rays and NG. Kaul and Bhan [18] also reported higher mutagenic efficiency for EMS.

Among the various treatments used in the present study, the most effective and efficient doses were 0.01% NMU and 0.05% NDEU, respectively. The results further indicated that a highly efficient mutagen need not be highly effective one also. A somewhat parallel conclusion was drawn by Konzak et al. [1] working with barley using a variety of alklyating agents. The genetic background of the material, the intracellular condition and perhaps cell cycle also play an important role in determining the effectiveness and efficiency of the mutagens.

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REFERENCES

1. C. F. Konzak, R. A. Nilan, J. Wagner and R. J. Foster. 1965. Efficient chemical mutagenesis. Rad. Bot. (Suppl.), **5:** 49–70.

2. R. A. Nilan, C. F. Konzak, J. Wagner and R. R. Legault. 1965. Effectiveness and efficiency of radiations for inducing genetic and cytogenetic changes. Rad. Bot., **5**: 71–89.

3. T. Gichner, 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-nitroso urea and N-ethyl-N-nitroso urea. Rad. Bot., **8**: 499–507.

4. V. N. Savin, M. S. Swaminathan and B. Sharma. 1968. Enhancement of chemically induced mutation frequency in barley through alteration in the duration of presoaking of seeds. Mutation Res., **6**: 101–107.

5. P. Dixit and D. K. Dubey. 1986. Mutagenic efficiency of gamma- rays, NMU and their combinations in lentil (*Lens culinaris* Med.) var. T. 36. Indian J. Genet., **46**(3): 501–505.

6. I. S. Solanki and B. Sharma. 1994. Mutagenic effectiveness and efficiency of gamma-rays, ethylene imine and N-nitroso-N-ethyl urea in *macrosperma* lentil (*Lens culinaris* Medik.). Indian J. Genet., **54**(1): 72–76.

7. N. B. Natal'in, S. B. Mosina and N. Chan Kuang. 1974. Mutational changes in rice. Genetika (USSR): **10**(2): 176–178.

8. H. Satoh and T. Omura. 1986. Mutagenesis in rice by treating fertilized egg cells with nitroso compounds. *In:* Rice Genetics. International Rice Research Institute, Manila: 707–717.

9. H. Gaul. 1960. Critical analysis of the methods for determining mutation frequency after seed treatment with mutagen Genet. Agrar., **12**: 297–318.

10. H. Gaul. 1970. Mutagen effects observed in first generation. *In:* Manual on Mutation Breeding. Technical Report Series No. 119. FAO/IAEA, Vienna: 85–98.

11. L. D. Vijendra Das and S. R. Sree Rangasamy. 1973. Effect of irradiation with gamma-rays on embryo sac in M_1 generation of rice. Curr. Sci., **42**: 437–438.

12. K. D. Sharma. 1986. Induced mutagenesis in rice. *In:* Rice Genetics. International Rice Research Institute, Manila: 679–695.

13. M. P. Singh and P. K. Sinha. 1986. Induced mutagenesis in native rices. *In:* Rice Genetics. International Rice Research Institute, Manila: 719–727.

14. L. D. Thanh and B. H. Trinh. 1986. Effects of dimethyl sulphate, ethylene imine, and gamma-rays on rice variability in M_2. *In:* Rice Genetics. International Rice Research Institute, Manila: 739–750.

15. A. J. Miah, G. Mustafa and A. M. Soomro. 1974. Mutations induced by chemical mutagens in rice (*O. sativa* L.). Andhra Agric. J., **19**: (1–2): 54–56.

16. G. M. Rao. 1983. Mutagenic efficiency, effectiveness and factors of effectiveness of physical and chemical mutagens in rice. Cytologia, **48**(3): 427–436.

17. E. A. Siddiq and M. S. Swaminathan. 1968. Enhanced mutation induction and recovery caused by nitroso-guanidine in *Oryza sativa*. Indian J. Genet., **28**(3): 297–300.

18. M. L. H. Kaul and A. K. Bhan. 1977. Mutagenic effectiveness and efficiency of EMS, DES and gamma-rays in rice. Theor. Appl. Genet., **50**: 242–246.