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A Comparison of Mutagenic Effectiveness and Efficiency of NMU and MNG in Sorghum

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Summary. A comparative study of the effectiveness and efficiency of NMU and MNG in relation to the effects in M1 plants and induction of mutations in M2 was made in a cultivated variety of *Sorghum*. There was a decrease in the values obtained in each of the biological criteria in the M1, namely germination, survival, seedling growth and seed fertility, with an increase in the concentration of NMU and MNG. Of the characters, survival following MNG treatments and seed fertility following NMU treatments showed the maximum reduction. NMU was not only effective in decreasing the mean of various characters in the M1, but also efficient in inducing a high frequency and wide spectrum of chlorophyll mutations in the M2 compared with MNG. Low concentrations were found to be more efficient than higher concentrations of NMU. The treated M2 population showed not only a decrease but also an increase in the mean height of plant and length of ear compared with the untreated control population. NMU caused a greater decrease in the mean of both characters and induced greater variability in the length of ear than did MNG treatment.

The mutagenic and carcinogenic effects of N-nitroso compounds are assumed to be caused by their decomposition in vivo to diazoalkanes and by the alkylation ability of the diazoalkanes (Lee et al., 1964; Magee and Schoental, 1964; Marquardt et al., 1964). Of the various N-nitroso compounds, N-nitroso methyl urea (NMU) and N-methyl-N-nitroso-N′-nitrosoguanidine (MNG) have proved to be highly efficient mutagens. Previous studies on the action of NMU and MNG in barley, *Arabidopsis* and rice (Ehrenberg and Gichner, 1967; Gichner and Veleminsky, 1967; Müller and Gichner, 1964; Veleminsky et al., 1967; Swaminathan et al., 1968) have shown that these chemical mutagens differ in their effects on various biological characters. There is contradictory evidence as to the mutagenic efficiency of MNG in higher plants. MNG has been reported to be more toxic than mutagenic in barley (Ehrenberg and Gichner, 1967). On the other hand, Swaminathan et al. (1968) using rice, Gichner (1965), Gichner and Veleminsky (1967), Müller and Gichner (1964) and Veleminsky et al. (1967) in *Arabidopsis*, Prasad et al. (1967) in barley, and Kempenna et al. (1969) in wheat reported that MNG is a highly potent mutagen. Basic information on sensitivity and mutation induction would be very useful in the improvement of *Sorghum* through mutation breeding; the improvement of the *Sorghum* crop has so far been accomplished largely by hybridization. The present study was undertaken to elicit more information on the action of NMU and MNG on various biological characters in the M1 generation and on the induction of chlorophyll and polygenic mutations in the M2 generation.

Materials and methods

The variety co. 18 (*Sorghum subglabrescens*) was used in the present study. It is an important grain-cum-fodder type which is grown extensively in the state of Tamil Nadu in India. Dry and well filled seeds of uniform size were pre-soaked in water for four hours and treated with NMU and MNG for eight hours. The chemicals used in the present study were obtained from K and K laboratories. The chemical solutions were freshly prepared in water just before the treatment. All the treatments were administered at 26 ± 1 °C. The solutions were shaken continuously and the volume of the solution was maintained at ten times that of the seeds. The seeds soaked in water for 12 hours were sown as control. After treatment, the seeds were washed in running water for 30 min. and sown immediately in germination trays and in the field. Data were gathered from three replications laid out in randomized blocks. The M1 was studied for the following characteristics to compare the biological effects of NMU and MNG: germination was recorded from the second day onwards, while the data on emergence of the first leaf were collected from the fourth day; survival was recorded 30 days after sowing; the lengths of the primary root and coleoptile were taken from four-day-old seedlings; the height of seedlings was measured on six-day-old seedlings. A total of seventy five seedlings per dose was considered for the measurements. All these observations were recorded from the seedlings grown in germination trays. Seed fertility was estimated from 40 M1 plants chosen at random in each treatment under field conditions. The plants in the M1 generation were bagged to ensure self-fertilization and the seeds were gathered separately from each M1 plant to raise M2 population. The variety used is of non-tillering type and hence it bears a single ear on the main culm. Hand sowing was adopted uniformly throughout the experiments, using a spacing of 6 inches between plants and 18 inches between rows. The M2 population was divided into two groups. The first group consisted of macro-mutations, as suggested by Gaul (1965), which were studied separately, while the other group included plants without any visible phenotypic changes. Chlorophyll mutations were scored in the first group when the seedlings were 8 to 15 days old. Variability for two quantitative characters, height of plant and length of ear, was studied in the second group of plants. The M2 data were gathered from three replications laid out in randomized blocks. Mutagenic effectiveness and efficiency were estimated using the formulae adopted by Konzak *et al.* (1965).

Results

The data on the effects of NMU and MNG on various biological characters in the M1 are presented in Fig. 1 and 2. There was a decrease in the percentage of germination, survival, length of root and coleoptile and height of seedlings with increasing doses of chemical mutagens. A stimulatory effect was apparent at the lower concentrations, while at higher doses of NMU there was marked delay in the period of germination and emergence of first leaf (Fig. 1). Seed fertility was found to decrease with increased concentrations of NMU and MNG. Of the various characters, survival following MNG treatments and seed fertility following NMU treatments showed the maximum reduction. It is also evident from the data that NMU was more effective than MNG in decreasing the mean of various characters. For example, LD.50 survival was 1.36 mM in MNG, while it appeared to be below 0.288 mM in NMU. For seed fertility, the LD. 25 was 2.72 mM with MNG treatment, whereas it was 0.048 mM with NMU.

In the M2 generation, chlorophyll mutations were scored on an M1 and M2 plant basis following treatment with NMU and MNG (Table 1). The data show

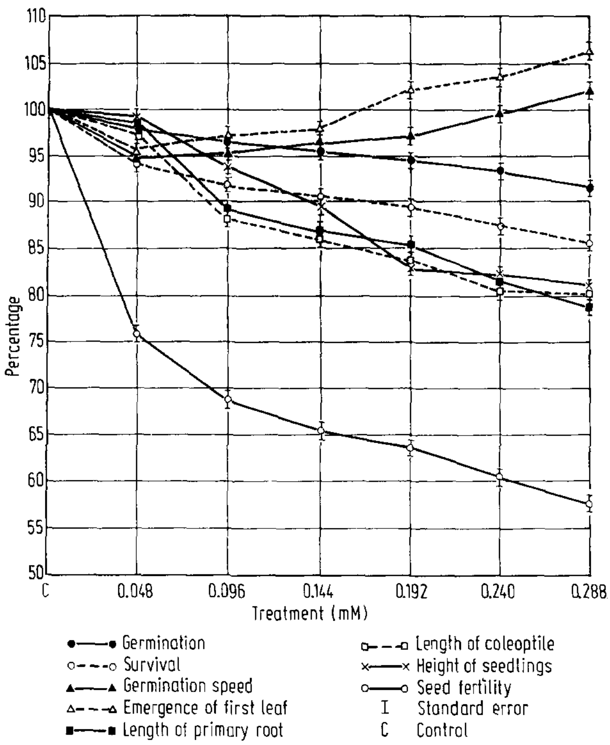

Fig. 1. Effect of NMU on various biological characters in the M1

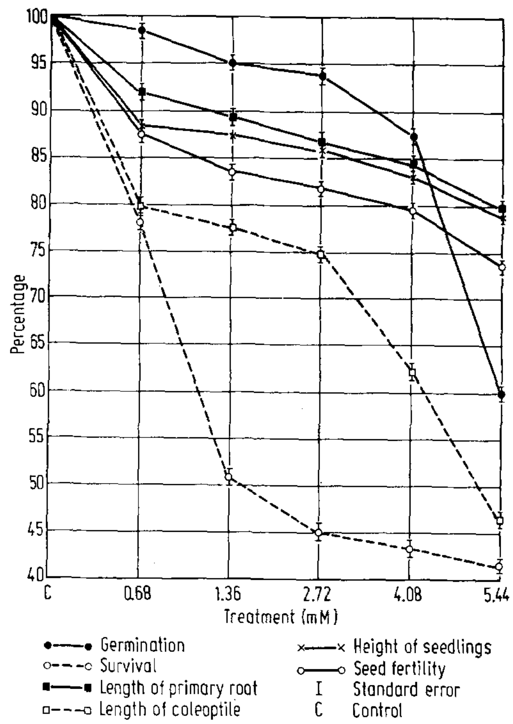

Fig. 2. Effect of MNG on various biological characters in the M1

Table 1. *Frequency of chlorophyll mutations in M2 after treatments with NMU and MNG*

Treatment	Number of M1 plants scored	Number of M1 plants segregating M2	Number of M2 plants scored in M2	Number of mutants in M2	Mutation Frequency (%)	
					M1 plant basis	M2 plant basis
Control	290	0	5120	0	0	0
NMU						
0.048 mM	244	16	13092	78	6.56	0.59
0.096 mM	235	30	12335	129	12.77	1.05
0.144 mM	264	36	6918	54	13.64	0.78
0.192 mM	199	40	9972	203	20.10	2.04
0.240 mM	240	50	4770	54	20.83	1.13
0.288 mM	204	42	4896	96	20.59	1.96
MNG						
0.68 mM	142	3	9452	5	2.11	0.05
1.36 mM	174	12	13995	24	6.89	0.17

Table 2. *Spectrum of chlorophyll mutations in M2 following treatments with NMU*

Treatment	Relative percentage of mutants							
	Albina	*Viridis*	*Xantha*	*Chlorina*	*Striata*	*Tigrina*	*Alboviridis*	*Xanthalba*
0.048 mM	12.8 (10)*	59.0 (46)	2.6 (2)	0	0	0	25.6 (20)	0
0.096 mM	4.6 (6)	23.4 (30)	0	4.6 (6)	67.4 (87)	0	0	0
0.144 mM	17.1 (9)	55.4 (30)	21.8 (12)	0	5.7 (3)	0	0	0
0.195 mM	58.0 (117)	9.8 (20)	0	0	0	0	27.8 (57)	4.4 (9)
0.240 mM	22.2 (12)	38.9 (21)	0	0	0	3.33 (18)	5.6 (3)	0
0.288 mM	46.9 (45)	50.0 (48)	0	0	3.1 (3)	0	0	0

* Number of mutants are given in parenthese

that there was an increase in the frequency of mutations with an increase in the dose of NMU, when measured on the M1 plant basis, while on the M2 plant basis, the middle dose gave the highest mutation frequency. The rate of chlorophyll mutations was found to be highest in the NMU treatments. A wide spectrum of mutations was noticed following NMU treatments, but there were only four types, *albina* and *xantha* in 0.68 mM and *tigrina* and *striata* in 1.36 mM in the MNG treatments. The data on spectrum of mutations in the NMU treatments (Table 2) revealed that there was no relationship between the number of mutant types and the dose of mutagen. However, the proportion of each of the mutant types varied with the different doses. *Albina* and *viridis* occurred in greater proportions than the rest of the mutant types.

The estimates of mutagenic effectiveness and efficiency of NMU and MNG are presented in Table 3. The mutagenic effectiveness of NMU treatment was found to be several times higher than that of MNG. The mutagenic efficiency was estimated on the basis of lethality, injury and sterility. Of the estimates, those on the sterility basis yielded very low values compared with those based on survival and injury following NMU treatments. The mutagenic efficiency was far higher following NMU than after MNG treatments.

The effect of NMU and MNG on polygenic traits, height of plant and length of ear, were studied in the M2 generation (Fig. 3 and 4). The treated population showed not only a decrease but also an increase in the mean height of plant and length of ear. The data further revealed that there was no relationship between the dose and the mean of the character. NMU caused a greater reduction in the mean of the two characters than did MNG. The phenotypic distribution of frequencies in the treated population showed a shift to the left of the control, indicating the variability to be negative. The magnitude of variation was found to be higher in the treated population than in the untreated population. NMU induced the maximum variation in the length of ear.

Table 3. *Mutagenic effectiveness and efficiency of NMU and MNG*

Treatment	Mutagenic effectiveness	Mutagenic efficiency		
	$\frac{M}{C \times t}$	$\frac{M}{L}$	$\frac{M}{I}$	$\frac{M}{S}$
NMU				
0.048 mM	1.224	0.087	0.522	0.019
0.096 mM	1.510	0.153	0.197	0.038
0.144 mM	0.677	0.089	0.086	0.023
0.192 mM	1.328	0.206	0.129	0.058
0.240 mM	0.588	0.099	0.067	0.029
0.288 mM	0.851	0.157	0.109	0.015
MNG				
0.68 mM	0.009	0.002	0.004	0.004
1.36 mM	0.015	0.003	0.001	0.001

M = Frequency of chlorophyll mutations in M2 (M2 plant basis)
C = Concentration of chemical.
t = duration of treatment.
L = Lethality or survival reduction of seedlings in M1.
I = Injury or seedling height reduction in M1.
S = Seed sterility in M1 plants.

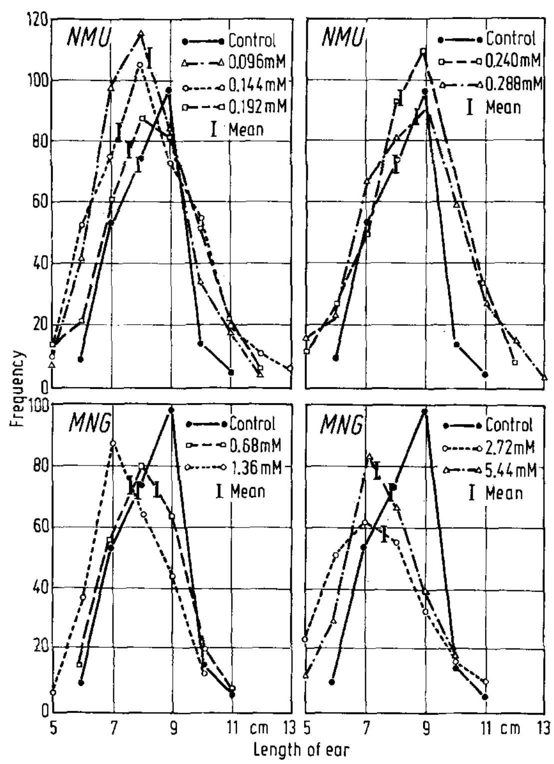

Fig. 3. Phenotypical distribution of length of ear of control and M2 plants

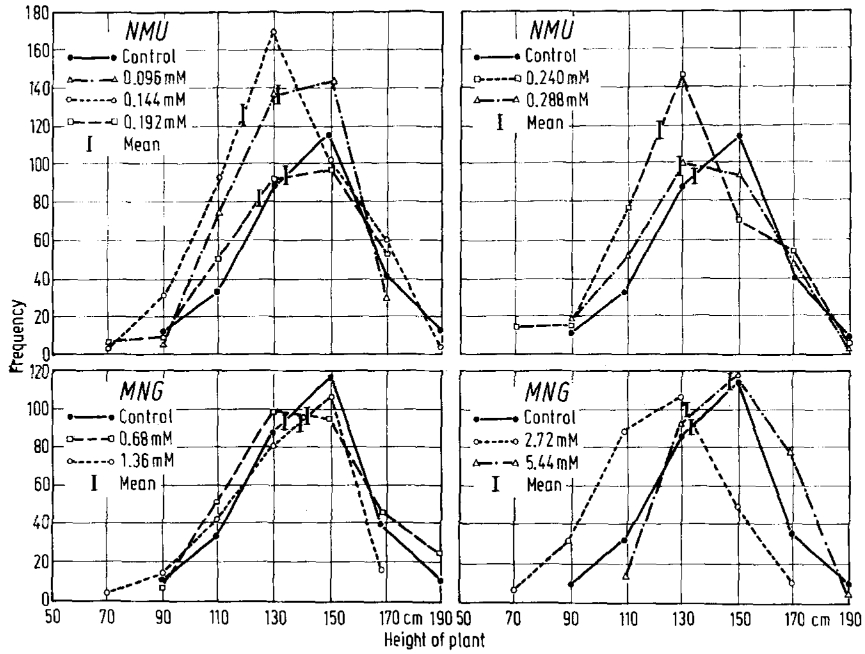

Fig. 4. Phenotypical distribution of plant height of control and M2 plants

MNG were not noticed in *Arabidopsis* (Veleminsky et al., 1967). Such differences in the effects of mutagens on different material might be due to the seed metabolism and onset of DNA synthesis. Further, it is also evident that survival following MNG treatment and seed fertility after NMU treatment showed a greater reduction than the other characters. NMU was found to be several times more effective than MNG in decreasing the mean of various characters.

The frequency of chlorophyll mutations showed a saturation effect following treatment with higher concentrations of NMU, when the rate of mutation was computed on M2 plant basis. This effect following treatment with mutagens in the M2 has been reported in several crop plants (Ehrenberg and Nybom, 1954; Matsuo and Yamaguchi 1962; Wellensiek, 1965). It has been attributed to the rigour of both diplontic and haplontic selection in the biological material (Swaminathan, 1961). NMU induced a very high frequency and a wide spectrum of chlorophyll mutations compared with MNG. This was also true when comparing their mutagenic effectiveness and efficiency as calculated by the scheme proposed by Konzak et al. (1965).

Ehrenberg and Gichner (1967) found no mutations in the M2 of barley following MNG treatment, while mutations segregated after treatment with NMU. Veleminsky et al. (1967) have correlated the differences in the action of NMU and MNG on various M1 characters with mutations in the M2. However, MNG proved to be a highly efficient mutagen in *Arabidopsis* (Gichner, 1965; Gichner and Veleminsky, 1967; Müller and Gichner, 1964; Veleminsky et al., 1967), rice (Swaminathan et al., 1968), barley (Prasad et al., 1967) and wheat (Kempenna et al., 1969).

In general, the treatments with low and medium concentrations of NMU were more effective and efficient, as measured on the bases of lethality, injury and sterility, than were the treatments with higher concentrations. The reason seems to be related to the fact that lethality, injury and sterility increase with mutagen concentration at a faster rate than mutations (Konzak et al. 1965). The efficiency of a mutagenic agent is a complex question, as it not only depends on the reactivity of the agent with the material and on its applicability to the biological system, but also on the degree to which physiological damage, chromosomal aberrations and sterility are induced in addition to mutations. Kaul (1969) and

Discussion

Mutagenic sensitivity is known to be influenced by a variety of factors, of which the type of mutagen and dose, moisture content of the seed, treatment conditions, ploidy, stage of development and genotype of the material are important. In comparing the effects of NMU and MNG, reduction of germination, survival, growth of primary root, coleoptile and seedlings, seed fertility and the delay in the emergence of leaf in the M1, and induction of chlorophyll and polygenic mutations in the M2, were taken as the main indices for overall response.

With an increase in the concentration of NMU and MNG, the values obtained in each of the biological criteria in the M1 decreased. Similar responses to mutagenic treatment have been reported in different crop plants. Stimulatory effects on the period of germination and emergence of first leaf were noticed following treatment with lower doses of NMU. There were striking differences in the action of NMU and MNG on various biological criteria. For example, there was no remarkable decrease in the percentage of germination following NMU treatment, unlike survival, growth of root, coleoptile and seedlings and seed fertility, while after treatment with MNG all the characters including germination showed a gradual decrease with increasing doses of mutagen. A similar situation has been reported in barley (Veleminsky et al., 1967), where the percentage germination decreased parallel with decreasing seedling height following MNG treatment, while with NMU the inhibition of germination was noticed after the doses inducing the maximum reduction in seedling height. However, such differences in the action of NMU and

Veleminsky and Gichner (1970) pointed out that the effectiveness and efficiency of nitroso amides were greatly influenced by pH and temperature during treatment.

A comparison of the effects of NMU and MNG on quantitative characters in the M2 revealed that NMU was more effective than MNG in decreasing the mean height of plant and the length of ear. The treated population showed low as well as high mean values compared with the untreated control population. Decreases in the mean of various quantitative characters in the treated population in M2 and subsequent generations have been demonstrated by Gregory (1956) in peanuts and Rawlings et al. (1958) in soya beans. Scossiroli et al. (1965) found a decrease in the means in M1 and M2 following irradiation in *Triticum* species and concluded that this change was a result of the elimination of undesirable genes and lethals after selfing. With the changes in the mean, the chemical treatments induced new genetic variability in the M2. The phenotypic distribution of frequencies for plant height and length of ear in the treated M2 plants showed a shift to the left of the control indicating the variability to be negative. However, the magnitude of variation was greater in the treated population compared with the control. NMU caused more variability in the length of ear than did MNG. Previous studies in rice (Oka et al., 1958; Kao et al., 1960; Matsuo and Onozowa, 1961), soyabeans (Rawlings et al., 1958; Williams and Hanway, 1961), wheat (Scossiroli et al., 1965; Swaminathan, 1963; Borojevic, 1966), barley (Gaul et al., 1969), *Arabidopsis* (Brock, 1965) and oats (Krull and Frey, 1961) revealed that mutagenic treatments induce substantial genetic variability in the M2 and subsequent generations. The results show that NMU, as well as being highly efficient in inducing chlorophyll mutations in relation to M1 effects, was also more potent than MNG in inducing genetic variability in quantitative traits.

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