

## GENETICS VARIABILITY IN MAIZE (*ZEa MAYS* L.) INDUCED BY MUTAGENS

### I. THE EFFECT OF COMBINED DOSES OF N-METHYL-N-NITROSOUREA, SODIUM AZIDE AND GAMMA RAYS<sup>1</sup>

JAN OLEJNICZAK<sup>2</sup>

Institute of Plant Genetics, Polish Academy of Sciences, Poznań

**Summary.** The action of combined doses of SA+MNUA and MNUA+gamma rays resulted in a marked increase in the number of chromosome aberrations in the root tips, as compared to separately acting mutagens. Sodium azide did not induce aberrations, and MNUA and gamma rays induced a relatively small number of aberrations. Singly acting mutagens caused small seedling injuries of  $M_1$  plants, whereas seedling injuries of  $M_1$  plants were found to increase and decrease, when combined doses of mutagens were applied. The highest level of seedling injuries was detected in the case of SA+ gamma rays and MNUA+gamma rays interaction, and the lowest — in the case of gamma rays+SA and MNUA+SA interaction.

Among the applied mutagen combinations the largest frequency of chlorophyll mutations in  $M_2$  was induced by SA. The combined mutagen doses did not cause an increase in the mutation number in comparison to singly acting mutagens.

A mutagenic effect of gamma rays and that of the chemomutagen MNUA on higher plants have been known for many years, and recently, a high mutagenic effectiveness of sodium azide has been noted. The frequency of mutations may also increase interaction of chemical and physical mutagens. Mutation methods are used in basic and breeding works mainly with self-pollinating and rarely with cross-pollinating plants.

The purpose of the present paper was to determine the effect of interaction of N-methyl-N-nitrosourea, sodium azide and gamma rays on the size of seedling injuries of  $M_1$  plants of the maize line S-615 and on the frequency of chlorophyll mutations in  $M_2$  plants.

#### MATERIAL AND METHODS

The material for the present studies consisted of the corn seeds of the maize inbred line S-615 (*Zea mays* L.) obtained at the Experimental Department of Plant Breeding and Acclimatization in Smolice. Healthy and well-developed seeds were

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<sup>2</sup> Dr. Present address: ul. Strzeszyńska 30/36, 60 - 479 Poznań, Poland.

exposed to the action of two chemical mutagens and gamma rays in the following combinations:

- 1) control —  $H_2O$ ,
- 2) control —  $H_2O$  — buffer,
- 3) sodium azide (SA) — 0.7 mM,
- 4) N-methyl-N-nitrosourea (MNUA) — 0.8 mM,
- 5) gamma rays ( $\gamma$ ) — 7 kR,
- 6) SA+MNUA,
- 7) MNUA+SA,
- 8) gamma rays+SA,
- 9) SA+gamma rays,
- 10) gamma rays+MNUA,
- 11) MNUA+gamma rays.

Corn seeds were presoaked in distilled water for 12 hours at 24°C. The SA solution was prepared in phosphate buffer (0.025 M), pH 3, whereas MNUA was dissolved in distilled water, at pH 5.6. The grains were gamma-radiated (at the Laboratory of Isotopes, Academy of Agriculture in Poznań). The grains were treated with chemomutagens for 3 hours at 24°C, after which they were rinsed under running water.

Mutagen-treated seeds ready for cytological analyses were sown in Petri dishes. Germinating grains were fixed in aceto-alcohol (1:3) at 5 h-intervals from 31 to 78 hours of germination. Cytological observations of chromosome aberrations (bridges and fragments) at anaphase were made on smashed root tips prepared by Feulgen method.

In order to estimate the effect of mutagens on the growth of roots and seedling the treated seeds were placed in filter rollers in three replications at 24°C and additionally lightened for 18 hours/day. Fourteen days after the establishment of the experiment the root length and seedling height were measured. Besides that, mutagen-treated grains were also sown in the glasshouse. 7 days after the germination rate was assessed and 14 days after — the plant height was measured.

For comparison of the effect of mutagen combinations on the complex of seedling injuries (root length, seedling height, germination and plant height) as compared to the control, Mahalanobis' distances were used (Caliński, Kaczmarek 1969).

Mutagen-treated seeds were sown in the field at the space of  $15 \times 75$  cm in three replications by the method of random blocks, 280 grains in each replication, to obtain  $M_2$  generation. Two weeks after the germination in the control plants the germination rate on the plots was estimated. The first measurement of the plant height was made at the phase of 4 leaves and the second — before harvest. 30 random plants from each combination selected before were then self-pollinated during flowering. Before harvesting the plant survival was measured (i.e. the number of plants containing at least one grain on cross-pollinated cobs, was determined).

After harvesting inbreedingly pollinated cobs, the number and weight of the corn seeds were estimated. The frequency of chlorophyll mutations were determined on  $M_2$  plants according to Gustafsson's simplified classification.

For all the studied elements of seedling injuries (germination, plant height (I and II measurements), survival, seed number per cob seed weight per cob) of  $M_1$  plants and chlorophyll mutations of  $M_2$  generation contrasts were made between the control and mutagen combinations. For individual mutagens and the control form, Mahalanobis' distances, the significance of which was studied through the comparison with critical distances at  $\alpha=0.05, 0.01$ , were calculated.

## RESULTS

Depending on the kind and combination of mutagens, the observed percentage of chromosome aberrations was different (Tab. 1). The dose of 0.7 mM of sodium azide did not cause any aberration at all. A small number of chromosome aberrations

Table 1. Chromosomal aberrations after combined treatments with SA, MNUA and gamma rays

Combinations	Aberrant anaphases [%]	Time after treatments [h]
Control — $H_2O$	—	—
Control — $H_2O$ — buffer	—	—
Sodium azide (0.7 mM) SA	—	—
MNUA (0.8 mM)	6.5	48
SA (0.7 mM) + MNUA (0.8 mM)	10.0	53
MNUA (0.8 mM) + SA (0.7 mM)	4.5	58
Gamma rays — 7 kR	18.0	63
Gamma rays (7 kR) + SA (0.7 mM)	14.2	63
SA (0.7 mM) + gamma rays (7 kR)	13.5	78
(7 kR) + MNUA (0.8 mM)	14.0	73
MNUA (0.8 mM) + (7 kR)	25.0	78

tions was detected after treatment with 0.8 mM MNUA solution, but a large number — after the effect of a 7 kR dose of gamma rays. The application of a 0.7 mM SA dose together with a 0.8 mM MNUA dose induced a marked increase in the number of aberrations, as compared to a 0.8 mM MNUA dose.

The application of sodium azide after MNUA caused an insignificant reduction of aberration number in relation to the concentration of 0.8 mM MNUA. The dose of gamma rays with sodium azide pronouncedly decreased the number of aberrations in comparison to a 7 kR dose of gamma rays. The dose of MNUA applied after the effect of gamma rays caused a decrease in the number of aberrations in relation to a singly acting physical factor (gamma rays). Seed treatment with 0.8 mM of MNUA before the action of a physical factor increased the number of chromosome aberrations as compared to a 7 kR dose.

Mahalanobis' distances (Diagram 1) for the complex of seedling injuries (root length, seedling height, germination and plant height) as compared to the control generally showed that the applied mutagens and their combinations induced significant changes in the complex of the studied characters. An exception is the buffer

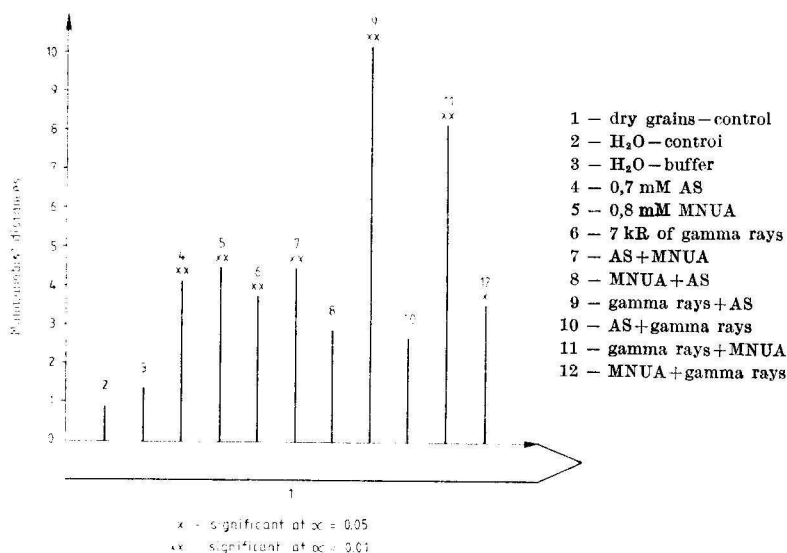


Fig. 1. Mahalanobis' distances for seedling injuries in laboratory and Eggebrecht's tests between the control form and combinations of mutagens

solution, the combination of 0.8 mM MNUA + 0.7 mM SA and 0.7 mM SA + 7 kR gamma rays. A particularly high level of seedling injuries was found in the case of interaction between gamma rays and chemical mutagens.

An analysis of contrasts of germination between the standard and mutagen combinations (Tab. 2) indicates that the combined dose of 0.8 mM MNUA + 0.7 mM SA induced significant stimulation of germination and that the combination of 0.7 mM SA + 7 kR and 7 kR + 0.7 mM SA definitely decreased the number of germinating plants. The first measurement of the plant height performed at 4-leaf

Table 2. An estimate of contrasts of the control (H<sub>2</sub>O) with different combinations of SA, MNUA and gamma rays for seedling injuries of  $M_1$  generation and frequency of chlorophyll mutations of  $M_2$

Contrast	Contrast values						
	germination	Plant height I measurement	plant height II measurement	survival	number of set seeds per cob	grain weight per cob	frequency of chlorophyll $M_2$ plants
(K) - K (buffer)	-1.33	-7.81*	-5.42	-1.67	67.26*	0.74	-0.63
(K) - 0.7 mM SA	1.67	-6.86	-0.15	4.33	30.21	-7.11	-2.79*
(K) - 0.8 mM MNUA	-6.00	0.63	18.73*	8.67	97.34*	6.25	-2.47*
(K) - 7 kR gamma rays	1.33	2.75	5.95	9.67	70.53*	4.68	-1.96*
(K) - SA + MNUA	4.00	1.85	25.03*	22.00*	116.39*	9.13*	-2.05*
(K) - MNUA + SA	-10.00*	-4.65	20.14*	1.00	88.32*	4.72	-1.40*
(K) - gamma rays + SA	4.00	1.87	9.81	9.67	93.47*	7.83	-2.24*
(K) - SA + gamma rays	31.00*	13.10*	29.61*	45.67*	126.33*	10.32*	-1.92*
(K) - gamma rays + MNUA	-1.33	8.54*	28.94*	28.00*	122.99*	10.42*	-1.21*
(K) - MNUA + gamma rays	20.33*	6.09	27.11*	35.00*	108.24*	8.71	-1.55*

\* Contrast significant at  $\alpha = 0.05$

stage showed a significant stimulating effect of the buffer solution on the plant height, whereas the effect of combined doses of 0.7 mM SA+7 kR and 7 kR+0.7 mM SA caused a clear reduction of the plant height in relation to the control.

Measurements made just before harvesting showed that almost in all the cases a significant reduction of the seedling height was induced by mutagens. Nearly all the combined mutagen doses, except 7 kR+0.7 mM SA and 0.8 mM MNUA+0.7 mM SA decisively caused a decrease in the survival of  $M_1$  plants. Each of separately applied mutagens did not decrease markedly the survival of  $M_1$  plants. The mean number of set seeds per cob significantly decreased in all mutagen combinations, except 0.7 mM SA dose. Out of many mutagen combinations only the doses of 0.7 mM SA+0.8 mM MNUA, 0.7 mM SA+7 kR and 7 kR+0.8 mM MNUA caused a marked decrease in the seed weight per cob in comparison to the control. The frequency of chlorophyll mutations of  $M_2$  plants (Tab. 2) showed a significant number of mutations after the action of all mutagen combinations. The highest frequency of mutations was detected after the action of 0.7 mM SA and 0.8 mM MNUA doses and 0.7 mM SA+0.8 mM MNUA and 0.7 mM SA+7 kR combinations. The combined mutagen doses did not induce a stimulating effect of chlorophyll mutations resulting from the action of single mutagens.

The obtained Mahalanobis' distances (Diagram 2) for the complex of seedling injuries (germination, plant height, survival, number of set grains per cob and grain weight per cob) of  $M_1$  plants between the control and individual combinations show in all the cases significant differences in the response to the applied mutagens. The highest level of seedling injuries was observed after the action of the

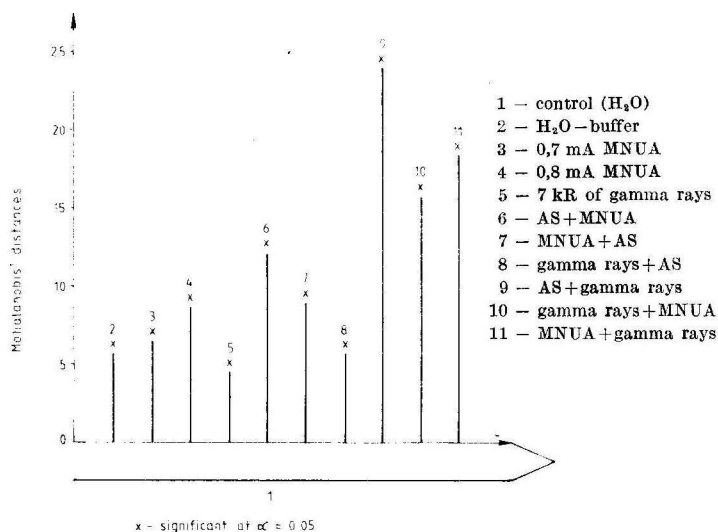


Fig. 2. Mahalanobis' distances for seedling injuries of  $M_1$  generation between the control form and individual combinations of mutagens

combined doses of gamma rays and MNUA and interaction of SA with gamma rays.

Sodium azide applied after gamma rays markedly reduced the size of seedling injuries in comparison with singly acting mutagens.

## DISCUSSION

Mutagenic means induce chromosome aberrations, mutations and seedling injuries of  $M_1$  plants. The last ones decrease the effectiveness of applied mutagens.

In the performed studies no chromosome aberrations were found after the action of sodium azide. Similar results were obtained by Sideris et al. (1969), Nilan et al. (1973), Kleinhols et al. (1974), Walther (1975), Niknejad (1976) and Sander et al. (1978). A small number of aberrations was obtained after application of a low MNUA dose. A small number of aberrations was also noticed by Giehn et al. (1968) and by Zaslomovich et al. (1967) — after treatment with low concentrations of MNUA. Significantly higher per cent of chromosome aberrations after treatment with MNUA concentrations was revealed in various cultivated plants by Künzel and Małuszyński (1968), Grzesik (1980), Tanoi Yamaguchi (1969), and in maize by Larchenko and Morgun (1974), Morgun and Larchenko (1978). In our studies, the dose of 7 kR gamma rays induced a significant percentage of aberrations in the root tips of maize. Sideris (1968) as well as Sideris et al. (1973) also observed a high percentage of aberrations after gamma radiation in barley. Sodium azide used in combined doses after MNUA caused a decrease in the number of aberrations in comparison to a single-acting MNUA dose. Tawin and Shkvarnikov (1979) found a decrease of aberrations by about 20% in wheat after a combined treatment of sodium azide and MNUA.

The combination SA+MNUA markedly increased the number of aberrations in maize. Combined doses of chemical mutagens with gamma rays had a significant influence on the number of aberrations in maize. MNUA and SA-treatment after the action of gamma rays reduced the number of aberrations, whereas a MNUA-treatment before the action of gamma rays caused an increase in the aberration number in maize. Similarly, Sideris et al. (1969) proved that sodium azide applied after the action of gamma rays markedly increased the number of aberrations in barley, while Heiner et al. (1960) and Mohan Rao (1972) in the case of action of dES with physical factors observed an increase in the number of aberrations in barley. Interaction of chemical mutagens with physical factors frequently yield contradictory results caused by different mechanisms of mutagen action (Mohan Rao 1972), whereas Hartley (1980) is of the opinion that an increase in the aberration number after the effect of combined mutagen doses is caused by inhibition of repair processes at the S phase or by inhibition of postreplication repairs.

The applied mutagens caused disturbances in the germination, growth and development of  $M_1$  plants. A synergistic effect of a combined treatment by gamma rays

and EMS on the number of emerged plants in barley was detected by Khalathar and Bhatia (1975) and by Singh et al. (1978), whereas Gichner et al. (1978) found no decrease in germination after MNUA+SA action. A reduction in the number of emerged plants after the effect of SA was observed by Hasegawa and Inoue (1980), Olejniczak et al. (1978a, 1978b) in barley, Popova and Nikolov (1975). Morgun, Larchenko (1975) and Olejniczak, Patyna (1981) observed reduction in the germination of maize plants after treatment with chemical mutagens.

The next symptom of mutagen action was a marked reduction of the height and survival of maize plants after treatment by both single and combined mutagen doses.

A similar phenomenon after the action of single mutagen doses was found by Olejniczak et al. (1978a, 1978b), Singh et al. (1979) and by Hasegawa, Inoue (1980) in barley. The effect of combined MNUA and SA doses on the plant height and survival was also studied by Konzak et al. (1975) and Gichner et al. (1978) in barley. Popova (1978), Popova, Nikolov (1979) and Olejniczak, Patyna (1981) observed reduction of the plant height and survival after treating maize grains with chemical mutagens.

The applied mutagens also reduced the number of set seeds and the seed weight per cob, particularly after the application of combined doses. A similar reduction in the seed weight after the effect of combined MNUA and SA doses was observed in barley by Konzak et al. (1975) and in soybean by Constantin et al. (1976) — after the action of combined doses of other mutagens. Sarwella and Gorgan (1967) report about a decrease in the seed number per cob in maize after gamma-ray treatment.

One of the genetic effects of mutagen action is the occurrence of chlorophyll mutations. Among the applied mutagens the largest frequency of chlorophyll mutations in maize was induced by sodium azide. Hibberd, Green (1978) and Olejniczak, Patyna (1981) displayed a similarly high mutagenicity of sodium azide in maize and Olejniczak et al. (1978a, 1978b), Singh and Olejniczak (1979), Singh et al. (1979, 1980) and Jende-Strid (1978) — in barley. The applied MNUA caused the occurrence of chlorophyll mutations in maize. A similar symptom of MNUA action in maize was detected in maize by Popova and Nikolov (1975), Popova (1978) and by Morgun and Larchenko (1978), whereas that in barley was revealed by Ehrenberg and Gichner (1967), Gichner et al. (1978) and by Konzak et al. (1975).

The dose of 7 kR of gamma rays induced a low per cent of chlorophyll mutations in maize in relation to the studied chemo-mutagens. A similar, weak mutagenicity of gamma rays as compared to chemical mutagens was observed by Brunner and Mikaelson (1971), Künzel (1971), Prasad (1972) and Nerkar (1977).

The obtained results indicate that combined mutagen doses did not increase the frequency of chlorophyll mutations with regard to singly acting mutagens. An interacting effect of combined mutagen doses was observed by Konzak et al. (1975), Mohan Rao (1972), Choudhary and Kaul (1976) in cultivated plants.



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## ZMIENNOŚĆ GENETYCZNA KUKURYDZY (*ZEА MAYS* L.) WYWOŁANA DZIAŁANIEM MUTAGENÓW

### I. EFEKT WSPÓŁDZIAŁANIA KOMBINOWANYCH DAWEK N-NITROZO-N-METYLO- MOCZNIKA, AZYDKU SODU I PROMIENI GAMMA

#### Streszczenie

W wyniku działania kombinowanych dawek SA+MNUA i MNUA+promienie gamma stwierdzono wyraźne zwiększenie liczby aberracji chromosomowych w komórkach stożków wzrostu korzeni w porównaniu z oddzielnie działającymi mutagenami. Azydek sodu nie wywołał aberracji, a MNUA i promienie gamma wywołały stosunkowo małą liczbę aberracji. Pojedynczo działające mutageny spowodowały niewielkie uszkodzenia somatyczne roślin  $M_1$ , natomiast przy zastosowaniu kombinowanych dawek mutagenów stwierdzono zarówno zwiększenie jak i zmniejszenie uszkodzeń somatycznych roślin  $M_1$ . Największy poziom uszkodzeń somatycznych wystąpił przy współdziałaniu SA+promienie gamma, MNUA+promienie gamma, a najmniejszy przy współdziałaniu promienie gamma+SA oraz MNUA+SA.

Spośród zastosowanych mutagenów największą częstotliwość mutacji chlorofilowych roślin w  $M_2$  wywołało działanie azydku sodu. Kombinowane dawki mutagenów nie spowodowały zwiększenia liczby mutacji w porównaniu z pojedynczo działającymi mutagenami.

## ГЕНЕТИЧЕСКАЯ ИЗМЕНЧИВОСТЬ КУКУРУЗЫ (*ZEА MAYS* L.) ВЫЗВАННАЯ ДЕЙСТВИЕМ МУТАГЕНОВ

### I. ЭФФЕКТ ВЗАИМОДЕЙСТВИЯ КОМБИНИРОВАННЫХ ДОЗ N-НИТРОЗО-N-МЕТИЛМОЧЕВИНЫ, АЗИДА НАТРИЯ И ГАММА ЛУЧЕЙ

#### Резюме

В результате воздействия комбинированными дозами SA+MNUA и MNUA+ $\gamma$  обнаружено явное увеличение числа хромосомных aberrаций в клетках конуса роста корней по сравнению с отдельно действующими мутагенами. Азид натрия не вызвал aberrаций, а MNUA и гамма лучи вызвали сравнительно небольшое число aberrаций.

Отдельно действующие мутагены вызвали небольшие соматические повреждения растений  $M_1$ , в то время как при применении комбинированных доз мутагенов обнаружено как увеличение, так и уменьшение соматических повреждений растений  $M_1$ . Наибольший уровень соматических повреждений выступил при взаимодействии SA+ $\gamma$ , MNUA+ $\gamma$ , а наименьший — при  $\gamma$ +SA и MNUA+SA.

Среди использованных комбинаций мутагенов наивысшую частоту хлорофилловых мутаций растений  $M_2$  причинил SA. Комбинированные дозы мутагенов не вызвали увеличения числа мутаций по сравнению с отдельно действующими мутагенами.