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GENETIC VARIABILITY IN MAIZE (*ZEA MAYS* L.) INDUCED BY MUTAGENS
II. VARIABILITY OF MORPHOLOGICAL CHARACTERISTICS AND YIELD STRUCTURE COMPONENTS IN THE M_2 AND M_3 PLANTS OF THE MAIZE LINE S-615 INDUCED BY COMBINED TREATMENTS OF SODIUM AZIDE N-METHYL-N-NITROSOUREA AND GAMMA RAYS[1]

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Summary. The applied chemical mutagens (SA, MNUA), gamma rays and their combinations have considerably increased the variability range of morphological characters and yield structure components. It was observed that the analysed trait variability is larger in M_3 than in M_2 generation.

The sequence of mutagen treatment in combined doses has markedly effected the variation range of morphological characters of M_3 plants. As a result of the mutagen action a number of morphological maize mutants have been obtained.

The mutation method has been widely used to induce genetic variation in breeding works for over ten years. The variation range of traits may be increased by the application of physical and chemical mutagenic agents. The interaction of various mutagens may also increase mutation effectiveness. The extension of the variation range of quantitative traits in a mutagen-treated population creates a larger possibility for selecting forms with desirable useful traits.

The purpose of the present paper was to determine the influence of combined treatments of SA, MNUA and gamma rays on variation of morphological characteristics and yield structure components of M_2 and M_3 maize plants.

MATERIAL AND METHODS

An object of the studies was the inbred line S-615 (*Zea mays* L.) bred at the Experimental Department of Plant Breeding and Acclimatization in Smolice by Docent Dr. hab. Królikowski. The line S-615 originated from the population variety Wigor. The methods of treatment and obtaining of M_1 were described in Part I of the studies (Olejniczak 1985). The obtained M_2 and M_3 grains were sown out in

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populations in the field at the space of 15 × 75 cm in three replications by the method of random blocks, 240 grains in each replication representing all inbreedingly pollinated plants. On the mature plants of M_2 and M_3 generations the following characters were studied: the plant height, the ear insertion height, length of the last (upper) internode, tassel length, the number of tassel branches, the length and breadth of the flag leaf (leaf index: length ×width ×0.75), the number of leaves and the number of cobs.

The biometrical description of the yield structure components concerned the following characters: the pith length, the pith diameter, the number of kernel rows per cob, the number of kernels per row, the pith weight, the kernel weight per cob, the kernel number per cob and the 1000-kernel weight.

The obtained data were statistically treated. For each combination as well as for the control combinations the variation coefficients of the studied characters were calculated. In order to present the position of mutagen combinations, described by a complex of the studied characters, graphically on a plane the method of canonical variables was used (Caliński, Kaczmarek 1973). For individual pairs of mutagen combinations Mahalanobis's distances were calculated and their significance was studied by comparing them to the control critical distances at the level of $\alpha=0.05$ and $\alpha=0.01$ (Caliński, Kaczmarek 1969). In addition, on the basis of Mahalanobis's distances a dendrite between the combinations was determined.

Grains from inbreedingly pollinated cobs, which were not sown in populations, were sown out in the field in cobrows at the space of 15 × 75 cm. Throughout the vegetation season morphological observations were made. On the basis of these observations some mutants were selected.

RESULTS

VARIATION ANALYSIS OF MORPHOLOGICAL CHARACTERISTICS AND YIELD STRUCTURE COMPONENTS OF M_3 PLANTS

Mutagens caused an increase in the variation coefficients (Table 1) of the following morphological plant characters: the plant height, the number of tassel branches, the number of shapely cobs per plant. The dose of 0.7 mM of SA caused an increase in the variation of the flag leaf index in comparison to the control form. The remaining analysed characters (the height of plants, number of cobs per plant, the length of the last internodes, the tassel length, the number of leaves per plant) were found to have a reduction of the variation range. An analysis of the yield structure components (Table 2) showes an increase in the variation of the following characters: the pith diameter, the kernel weight and kernel number per cob. Combined doses of gamma rays and MNUA also extended the variation of the 1000-kernel weight.

The position of mutagen combinations in the plane of canonical variables (Fig. 1) and the dendrite, indicate that the mutagen doses significantly differentiated the morphological characteristics of plants in relation to the control (except the

Table 1. Variation coefficients of morphological characters of M_2 and M_3 plants depend on treatments

Mutagens	Plant height		Ear insertion height		Last internode length		Tassel length		No. of tassel branches		Flag leaf index		No. of leaves per plant		No. of shapely cobs	
	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3
Control-H_2O	9.4	10.6	20.7	20.9	22.0	17.7	14.9	14.7	26.5	33.0	39.0	54.4	11.7	10.5	0.0	0.0
Control-buffer	13.1	9.1	22.3	17.5	19.2	17.9	11.0	11.7	25.2	34.9	38.7	40.8	10.9	9.7	30.2	14.5
0.7 mM SA	12.5	15.8	19.3	29.5	21.5	21.6	14.1	24.1	26.5	53.4	45.4	68.4	8.9	10.7	27.4	19.8
0.8 mM MNUA	9.5	21.9	19.6	35.8	17.5	24.4	17.0	27.4	33.8	50.5	35.9	50.6	13.7	11.6	36.9	0.0
7 kR gamma	10.0	16.4	22.2	28.3	20.5	10.2	11.9	27.5	36.2	47.9	30.2	66.0	10.5	9.0	26.3	17.5
0.7 mM SA+0.8 mM MNUA	9.9	20.3	23.3	33.1	18.0	32.1	13.3	32.0	31.6	69.4	33.6	74.8	10.8	13.5	32.6	14.5
0.8 mM MNUA+0.7 mM SA	9.4	17.1	17.7	28.6	16.6	24.6	12.0	29.0	35.3	64.3	35.8	61.4	12.7	11.0	32.1	14.5
7 kR+0.7 mM SA	9.4	17.5	16.5	41.0	17.6	23.0	10.4	24.0	28.1	52.4	39.0	76.1	9.4	12.3	18.4	0.0
0.7 mM SA+7 kR	11.0	22.4	19.9	29.4	10.8	24.3	18.8	24.5	29.7	65.2	28.5	61.2	8.6	8.0	32.2	0.0
7 kR+0.8 mM MNUA	7.4	16.8	20.6	33.0	16.6	21.9	11.5	25.8	31.5	72.7	33.9	81.2	8.5	10.0	32.0	0.0
0.8 mM MNUA+7 kR	14.1	18.4	24.6	36.0	15.7	19.9	14.6	24.4	32.5	73.7	38.8	73.7	9.5	12.7	30.9	25.0

Table 2. Variation coefficients of the yield structure traits of M_2 and M_3 plants depend on treatments

Mutagens	Pith length		Pith diameter		No. of kernel rows per cob		Kernel No. per row		Pith weight		Kernel weight per cob		Kernel No. per cob		1000-kernel weight	
	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3	M_2	M_3
Control-H_2O	15.2	16.3	7.1	6.8	12.6	16.8	12.0	26.2	35.1	24.8	36.2	43.5	32.3	45.8	18.7	24.1
Control-buffer	13.8	15.3	10.3	6.7	10.7	16.9	10.9	26.0	31.7	82.7	44.6	51.9	38.3	53.1	16.6	24.9
0.7 mM SA	14.2	19.4	11.1	9.7	17.5	18.7	12.6	33.7	31.5	31.5	40.6	60.8	38.3	63.0	14.3	27.9
0.8 mM MNUA	13.6	19.4	11.6	26.8	10.7	22.5	10.4	34.6	27.4	39.5	42.6	70.3	39.8	54.1	16.4	31.9
7 kR gamma	12.7	20.2	13.4	10.4	15.8	16.8	11.9	35.8	29.0	35.9	50.7	68.3	48.4	58.7	15.8	33.0
0.7 mM SA+0.8 mM MNUA	13.6	20.6	14.9	9.7	16.5	20.7	11.3	36.5	25.1	32.3	41.6	80.4	39.6	68.0	17.8	27.3
0.8 mM MNUA+0.7 mM SA	11.6	15.0	13.0	9.2	12.2	21.3	12.5	28.1	24.9	39.1	40.7	59.8	38.7	59.8	15.1	25.4
7 kR+0.7 mM SA	12.0	23.6	11.0	16.4	11.0	23.8	9.0	31.0	32.8	44.6	35.9	67.4	31.6	56.0	18.0	31.4
0.7 mM SA+7 kR	8.9	20.8	10.9	10.0	10.7	15.6	8.8	19.7	26.8	57.6	36.9	57.0	97.0	52.6	20.0	28.1
7 kR+0.8 mM MNUA	11.3	21.2	14.8	12.4	14.7	16.6	11.6	31.3	25.7	40.5	54.3	76.4	54.6	69.3	23.5	29.2
0.8 mM MNUA+7 kR	14.8	18.4	11.6	10.1	12.9	15.8	11.8	24.5	26.0	35.7	54.0	50.6	50.2	43.0	19.9	34.4

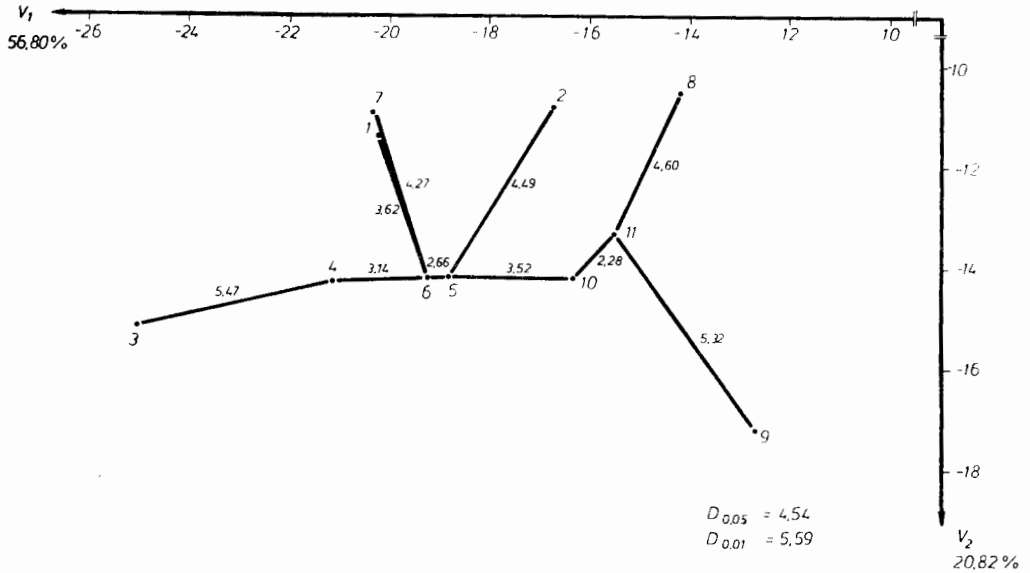

Fig. 1. The position of individual mutagen combinations in the plane of canonical variables and the dendrite between the combinations plotted on the basis of Mahalanobis's distances for the studied morphological characters of M_2 plants

1 – H_2O, *2* – H_2O-buffer, *3* – 0.7 mM SA, *4* – 0.8 m M MNUA, *5* – 7 kRγ, *6* – AS+MNUA, *7* – MNUA+AS, *8* – γ+SA, *9* – SA+γ, *10* – γ+MNUA, *11* – MNUA+γ

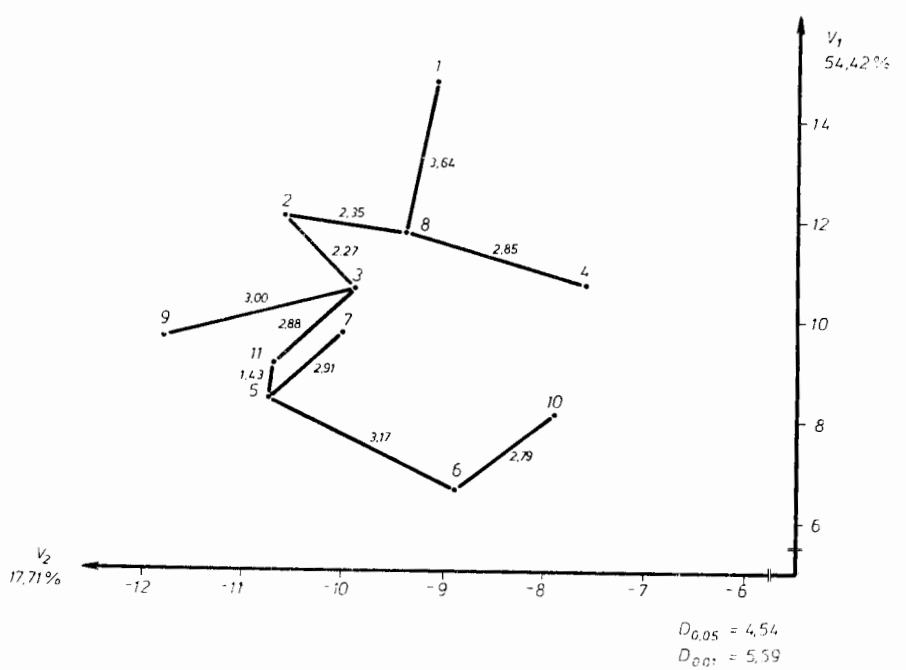

Fig. 2. The position of individual mutagen combinations in the plane of canonical variables and the dendrite between the combinations plotted on the basis of Mahalanobis's distances for the studied yield structure traits of M_2 plants*

* designations 1, 2, ..., 11 as in Diagram 1

combination 0.7 mM of SA+0.8 mM of MNUA). The sequence of action of gamma rays and SA in the combined doses markedly effected the morphological plant habit, which was not found in the case of the combined doses of MNUA and SA.

The yield structure components (Fig. 2) of M_2 plants presented in the system of canonical variables were significantly altered in relation to the control (except the doses of 7 kR gamma+0.7 mM SA). Significant differentiation was revealed with regard to the complex of the yield structure components after the action of sodium azide, N-methyl-N-nitrosourea and gamma rays. The sequence of the mutagen action in combined doses markedly effected the whole of the yield structure components of M_2 plants.

AN ANALYSIS OF VARIATION OF MORPHOLOGICAL CHARACTERS AND YIELD STRUCTURE COMPONENTS OF M_3 PLANTS

After the effect of all the doses of mutagens and their combinations, an increase in the variation range of morphological characters and yield structure components was found (Tables 1, 2). Variation of the studied traits induced by the interaction of SA+MNUA was larger than that in combination with MNUA+SA. Chemical mutagens SA and MNUA applied before gamma rays considerably increased the variation range of the yield structure components in comparison with the mutagens acting after a physical factor. It was observed that the habit of morphological plant characters was markedly altered in individual combinations as compared to the control.

The sequence of treatments with the mutagens SA and MNUA as well as with gamma rays and SA in combined doses caused no changes in morphological characters as a whole, whereas the sequence of using gamma rays with MNUA signi-

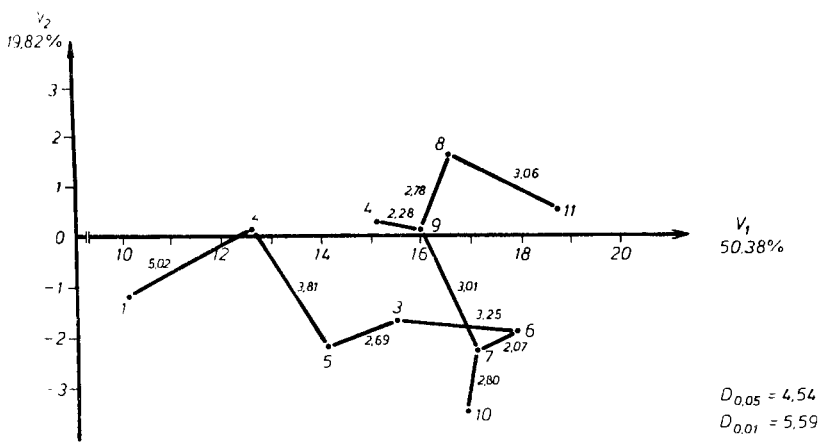

Fig. 3. The position of individual mutagen combinations in the plane of canonical variables and the dendrite between the combinations plotted on the basis of Mahalanobis's distances for the studied morphological characters of M_3 plants*

* designations 1, 2, ..., 11 as in Diagram 1

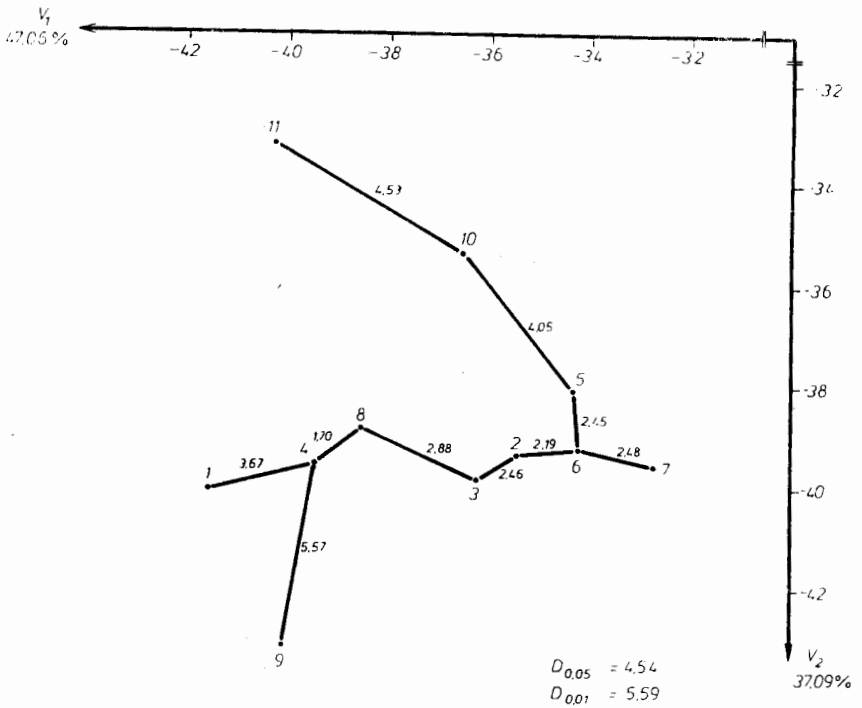

Fig. 4. The position of individual mutagen combinations in the plane of canonical variables and the dendrite between the combinations plotted on the basis of Mahalanobis's distances for the studied yield structure traits for M_3 plants*

* designations 1, 2, ..., 11 as in Diagram 1

ficantly influenced the complex of morphological characters (Fig. 3). Mutagens and their combinations considerably altered the whole of the yield structure components (Fig. 4) as compared to the control (except the MNUA dose). Combined treatments gamma rays with MNUA and of MNUA with gamma rays as well as that of SA+ +MNUA and MNUA+SA had no influence on the yield structure components. The yield structure components were pronouncedly altered in the case of interaction of gamma rays with SA and sodium azide with gamma rays. Over ten maize mutants with an altered habit of morphological characters mostly after a treatment with combined mutagen doses have been selected.

DISCUSSION

The use of mutation methods for increasing the variation range of quantitative traits was initiated by Gregory (1955) in peanuts and by Rawlings et al. (1958) in soybeans, by Oka et al. (1958) in rice and by Aestveit (1966) and Gupta (1969) in barley. Attempts to estimate variation of quantitative traits in a treated population were started by Scossiroli (1964) and Scossiroli et al. (1966) in wheat.

In the M_2 generation, particularly after the action of gamma rays with MNUA and the combination SA with MNUA, the variation range of the following characters was found to extend: the plant height, the number of auxiliary tassel branches and

the number of shapely cobs. Balint and Sutka (1966), using gamma rays in maize also obtained an increase in the variation range of M_2 and M_3 plant characters, such as the plant height, leaf number, the number of kernel rows per cob, the cob weight and the cob length. Mehandjiew (1975) observed the influence of the succeeding mutagens treatments in combined doses on the variation range of morphological characters in pea, and Abdalla and Hussein (1977) — in field bean.

Chemical mutagens applied before gamma rays in combined doses increased to a significant degree the variation of the yield structure components, as compared to those applied after a physical factor. In the studies of Abrams and Frey (1964) EMS+Nth (neutrons) interacted more effectively in extending the variation range of oats than they did in the combinations Nth+EMS, EMS+EMS and Nth and Nth.

A larger variation range of the studied traits was in the M_3 generation, as compared to that of M_2 generation. This results from segregation of mutated alleles. The obtained results agree with the observations conducted by of Bhatia and Swaminathan (1962) after treatment of wheat with X- and beta-rays.

The applied chemical mutagens increased the variation range of the studied characters in maize to a larger degree than d'd gamma rays. Ehrenberg et al. (1965) analysing the effect of X, EI and EMS on the quantitative traits of wheat in the M_2 and M_3 generations found a larger effectiveness of chemical mutagens than of X-rays. Similarly Conger et al. (1976), treating soybean seeds with various mutagens (gamma, Nth, EMS) found the largest variation of the studied traits after the action of EMS. Abrams and Frey (1964) report about a significant increase of the variation range of the plant height and seed weight in oats after the action of neutrons in comparison to the acting doses of EMS and P^{32}.

In the M_2 and M_3 generations of maize over ten mutants with an altered morphotype have been selected. Most of mutated forms were obtained from combined mutagen doses. Morgun and Larchenko (1975, 1978) as well as Popova (1978) using chemical mutagenesis obtained morphological mutants of maize. Neuffer and Coe (1978) treating maize pollen with EMS and NG obtained a high frequency of morphological mutations, and Shkvarnikov and Morgun (1964) displayed a high effectiveness of chemical mutagens for induction of maize mutants. Królikowski (unpublished) obtained new maize lines after the action of mutagens.

The obtained morphological mutants may be used in maize breeding and in analysis of evolutionary ways of the genus *Zea*.

REFERENCES

1. Aestveit K. (1966). Induced mutation in polygenic system. Hereditas, 56: 20 - 26.
2. Abdalla M. M., Hussein H. A. S. (1977). Effects in single combined treatment of gamma-rays and EMS on M_2 quantitative variation in *Vicia faba* L. Z. Pflanz., 78: 57 - 64.
3. Abrams R., Frey K. J. (1964). Variation in quantitative characters of oats (*Avena sativa*) after different mutagen treatment. Crop. Sci., 4: 163 - 168.
4. Balint A., Sutka J. (1966). Variability of quantitative characters in maize as affected by gamma irradiation. Acta Agrom. Acad. Sci. Hung., 15: 285 - 290.
5. Bhatia C. R., Swaminathan M. S. (1962). Induced polygenic variability of bread wheat and its bearing on selection procedures. Z. Pflanzucht., 48: 317 - 326.

6. Caliński T., Kaczmarek Z. (1969). A note on the calculation and use of the generalized distance between multivariate samples. Zesz. Nauk UAM, Geografia 8.
7. Caliński T., Kaczmarek Z. (1973). Metody kompleksowej analizy doświadczenia wielocechowego. Wydział V PAN i PTB, Warszawa.
8. Conger B. V., Skinner L. W., Skold L. N. (1976). Variability of components of yield induced in soybeans by seed treatment with gamma radiation, fission neutrons and ethylmethane sulfonate. Crop. Sci., 16: 233 - 236.
9. Ehrenberg L., Ekman G., Gustafsson A., Jansson G., Lundqvist U. (1965). Variation in quantitative and biochemical characters in barley after mutagenic treatments. Rad. Bot., 5: 477 - 490.
10. Gupta A. K. (1969). Induced polygenic mutation in diploid and tetraploid barley. Symp. Rad. and Radian Substances in Mutation Breeding, Sept. 26 - 29, 1969, Bombay.
11. Gregory W. C. (1955). X-ray breeding of peanuts (*Arachis hypogea* L.) Agrom. J., 47: 396 - 399.
12. Mehandjiew A. (1975). Podvyshenie geneticheskogo raznoobraziya u *Pisum sativum* z pomoshch'yu samostayatelnogo i kombinirovannogo primeneniya fizicheskikh i chimicheskikh mutagenov. Biuletyn IHAR 5 - 6: 39 - 43.
13. Morgun W. W., Larchenko E. A. (1975). Geneticheskaya aktivnost' nowykh nitrozosoedinenii na kukuruze. Genetika XI: 13 - 18.
14. Morgun W. W., Larchenko E. A. (1978). Izuchenie mutagennoi aktivnosti nitrozoalkilmochevin i diazoketonov na kukuruze. Tsitologiya i Genetika, 4: 331 - 337.
15. Neuffer M. G., Coe E. H. (1978). Paraffin oil technique for treating mature corn pollen with chemical mutagens. Maydica XXIII: 21 - 28.
16. Oka H., Hayashi J., Shiojiri J. (1958). Induced mutations of polygenes for quantitative characters in rice. J. Hered., 49: 11 - 14.
17. Popova J. (1978). Wleiyanie na dwukratno tretitante s etilmetansulfonat i N-nitroso-N--metyluretan wrchy mutabilnoctta na samoopraszeni linii carewica. Genetika i Selektsiya 1, 11: 78 - 81.
18. Rawlings J. O., Hanway D. J., Gardner C. O. (1958). Variation in quantitative characters of soybeans after seed irradiation. Agron. J., 50: 524 - 528.
19. Scossiroli R. E. (1964). Wheat mutagenesis in quantitative traits. In: Proc. Intern. Wheat Genetics Symp. 2nd, Lund, 85 - 101.
20. Scossiroli E. E., Palenzona D. L., Scossiroli-Pelegrini S. (1966). Studies on the induction of new genetic variability for quantitative traits by seed irradiation and its use for wheat improvement. In: Mutation in plant breeding IAEA, Vienna 1966, 197 - 229.
21. Shkvarnikov P. K., Morgun V. V. (1964). Mutations in maize induced by chemical mutagens. Polyploidy and Induced Mutations in Plant Breeding. IAEA, Vienna 1974, 295 - 303.

ZMIENNOŚĆ GENETYCZNA KUKURYDZY (*ZEA MAYS* L.) WYWOŁANA DZIAŁANIEM MUTAGENÓW
II. ZMIENNOŚĆ CECH MORFOLOGICZNYCH I CECH STRUKTURY PLONU ROŚLIN POKOLENIA M_2 I M_3 LINII S-615 WYWOŁANA WSPÓŁDZIAŁANIEM AZYDKU SODU, N-NITROZO-N-METYLOMOCZNIKA I PROMIENIAMI GAMMA

Streszczenie

Działanie mutagenów chemicznych (SA, MNUA), promieni gamma oraz ich kombinacji spowodowało rozszerzenie zakresu zmienności cech morfologicznych i cech struktury plonu roślin kukurydzy. Większą zmienność cech obserwowano w pokoleniu M_3 niż w M_2.

Zastosowanie mutagenów chemicznych, a następnie działanie promieniami gamma okazało się bardziej efektywne niż traktowanie mutagenami chemicznymi nasion uprzednio napromieniowanych. Ponadto działanie mutagenów chemicznych w większym stopniu niż promieni gamma poszerzało zakres zmienności badanych cech.

ГЕНЕТИЧЕСКАЯ ИЗМЕНЧИВОСТЬ КУКУРУЗЫ (*ZEA MAYS* L.) ВЫЗВАННАЯ ДЕЙСТВИЕМ МУТАГЕНОВ II. ИЗМЕНЧИВОСТЬ МОРФОЛОГИЧЕСКИХ ПРИЗНАКОВ И ЭЛЕМЕНТОВ СТРУКТУРЫ УРОЖАЙНОСТИ РАСТЕНИЙ В ПОКОЛЕНИЯХ M_2 и M_3 У ЛИНИИ КУКУРУЗЫ S-615, ВЫЗВАННАЯ ДЕЙСТВИЕМ АЗИДА НАТРИЯ, N-НИТРОЗО-N--МЕТИЛМОЧЕВИНЫ И ГАММА-ЛУЧЕЙ

Резюме

Химические мутагены (SA, MNUA), гамма-лучи и их комбинации увеличили предел изменчивости морфологических признаков и элементов урожайности растений кукурузы. Высшая изменчивость анализируемых признаков наблюдалась в поколении M_3 по сравнению с поколением M_2.

Применение мутагенов перед применением гамма-лучей оказалось более эффективным, чем после облучения зерна гамма-лучами. Кроме того, химические мутагены в большей степени расширяли предел изменчивости исследуемых признаков, чем гамма-лучи.