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EFFECTIVENESS OF FAST NEUTRONS IRRADIATION FOR THE STIMULATION AND INDUCTION OF GENETIC CHANGES IN SOYBEAN (*GLYCINE MAX* L. MERRILL) GENOME

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Abstract. Air-dry seeds of soybean cv. Warszawska were irradiated with fast neutrons (Nf) using the U-120 cyclotron (at the Institute of Nuclear Physics in Cracow) at the doses of 500, 1000, 1500 R. Additionally, each of the irradiation doses was combined with the selected effective chemical mutagen N-nitroso-N-methylurea - in three concentrations: 0.5, 1.5 and 2.5 mM, to evaluate synergistic effect of these two different mutagenic agents. The results showed some of protection effect of radiation on the level of somatic damage of soybean plants. In addition, the phenomenon of the "delaying effect" was noted, because the protection effect of fast neutron radiation in the combined treatments with chemomutagen was observed in the emergence and plant survival in the M_2 generation as well. From the point of view of genetic changes induced in the soybean genome, the most effective dose of fast neutron irradiation was 500 R. The number of soybean mutants with earlier ripening obtained (in comparison with original "mother" variety) at this irradiation dose was higher, than with the highest effective concentration of chemical mutagen (1.0 -1.5 mM MNUA).

Keywords: stimulation, fast neutrons, mutagenesis, soybean

INTRODUCTION

"In the Orient the soybean has a long history as a crop plant for human food and animal feed but elsewhere there has been significant production only in this century" [4]. In the Mediterranean region soybean is an important crop because it is a convenient a second crop [5]. Soybean seeds is a rich source of both oil and protein and it has become more and more important for the breeders in Poland as well [1,2]. However, the main difficulty in the cultivation of soybean in our country is period of vegetation that is too long. Shortening of this period with unchanged yield level becomes one of the important problems from breeders' point of view.

One of the ways to supplement the existing germplasm with additional variation and improving cultivars is mutation induction [3].

The aim of this work was to examine the reaction of plants to the combined treatment of fast neutrons (Nf) and selected effective chemical mutagen - N-nitroso-N-methylurea (MNUA) for the widening the genetic variability of soybean.

MATERIAL AND METHODS

The air-dry seeds of soybean cv. Warszawska were irradiated with fast neutrons (Nf) using the U-120 cyclotron (at the Institute of Nuclear Physics in Cracow) at the doses: 500, 1000, 1500 R. Additionally, each of the doses of irradiation was combined with three concentrations (0.5, 1.5 and 2.5 mM) of the selected, very effective chemical mutagens - N-nitroso-N-methylurea - to evaluate the synergistic effect of these two different mutagenic agents. Seeds were conditioned according to the method described by Sodkiewicz and Sodkiewicz [6]. After presoaking for 10 h in distillate water the seeds were treated with mutagen solution for 3 h at 24° C and washed in tap water.

The seeds of each treatment combination were sown in Petri dishes with quartz sand (50 seeds in 3 replications) - to check the level of somatic damage in the M_1 generation. The second part of the treated material was sown in the greenhouse (300 seeds in 3 replications). As the control combinations the seeds treated only with the chemical mutagen (MNUA) or distillate water were used.

In laboratory tests dynamic of soybean seeds germination was checked by counting the number of germinated seeds every 8 h, from 18 until 58 h after treatment.

The number of emerged plants and the size of soybean plants in each treatment combination was assessed on the material developed in the greenhouse after six weeks from sowing. On the basis of these data the per cent of reduction of emergence, growth and survival was evaluated in respect to control combination. Morphological observations and measurements of mature plants in the M_1 generation were made as well. The M_1 plants were harvested separately. In the experiment with M_2 generation seeds of each single M_1 plant were grown in individual row in a field trials.

To establish the extent of damage resulting from the treatment with Nf and MNUA, reduction of emergence, and survival rate was also observed in the M_2 generation. The M_2 plants of each treatment combination (all together 885 lines) were analysed for the different characters in their morphology (i.e. the plant high). Observations of phenologic phases of plant growth were also conducted during the whole vegetation period. The number of plants with earlier maturity in comparison with the plants of Warszawska cultivar was counted in each treatment combination.

RESULTS AND DISCUSSION

In the research work on inducing mutation, the following schedule is usually realised: the 1st step is optimalisation of doses of the mutagenic agents used based on laboratory and field tests and developing of the M_1 generation. In the M_1 generation the level of somatic damage produced by the mutagen used is examined. Genetic with mutagenic agent variability after treatment is usually analysed in the M_2 and M_3 generations.

The level of somatic damage produced by fast neutrons (Nf) and chemical mutagen (MNUA) in the M_1 generation was compared and also their synergistic effect was studied on the basis on the analysis of dynamic soybean seed germination. Data in Table 1 showed, that 18 h after treatment strong delay of germination was observed related to the concentration of a certain mutagen agent applied. In comparison with the control combination, reduction of germination in percent occurred mainly after chemical mutagen treatment or after combined treatment with both agents (Nf+MNUA) with higher doses of MNUA. Fast neutrons caused the smallest damage in germination, and in the first hours of germination after the dose of 500 R Nf even the stimulation effect was observed.

The effects observed in laboratory tests were confirmed in the greenhouse conditions. The irradiation of the seeds did not cause reduction of emergence, positively influenced the number of emerged plants, irrespective of the used doses. Contrary to the above treatment with MNUA caused reduction of plant emergency even with the lowest concentration of 0.5 mM. Positive interaction effects of the combined (Nf + MNUA) treatments on the M_1 plant emergency, was noted especially in the combination of Nf + 0.5 mM MNUA where the stimulation of emergency was seen (Table 2). Also, the influence of fast neutrons on the growth of treated plants resulted in stimulation at two of the used doses of Nf. Growth stimulation after MNUA treatment was observed only at the lowest concentration. The combined treatment of both mutagenic agents caused lower levels of growth and damage in the M_1 generation as expected from the individual effects of treatment of each mutagenic agent. The results showed a kind of protection effect of radiation on the level of somatic damage to soybean plants.

Table 1. Germination dynamic of soybean seeds cv. Warszawska after fast neutron (Nf) and N-nitroso-N-methylurea (MNUA) treatment

Combination of treatment	Number of hours after treatment					
	18	26	34	42	50	58
	Percentage of germinated seeds					
H_2O - Control	13.34	28.00	40.67	48.00	50.67	54.67
0.5 mM MNUA	7.34	22.67	37.34	46.00	54.67	56.66
1.5 mM MNUA	6.66	19.34	36.00	39.34	43.34	49.34
2.5 mM MNUA	4.67	16.00	26.00	40.00	45.34	46.67
500 R Nf	16.00	31.34	43.34	53.34	55.34	56.00
1000 R Nf	14.67	31.34	51.34	62.67	63.34	64.67
1500 R Nf	11.34	24.00	35.34	45.34	46.67	48.67
500 R Nf + 0.5 mM MNUA	12.00	30.00	43.34	50.00	55.34	56.66
500 R Nf +1.0 mM MNUA	10.67	28.00	50.67	60.67	64.00	64.67
500 R Nf +2.5 mM MNUA	4.67	27.34	40.00	53.34	57.34	57.34
1000 R Nf + 0.5 mM MNUA	12.67	28.67	53.34	64.00	64.67	68.67
1000 R Nf + 1.0 mM MNUA	12.67	35.34	48.67	58.00	63.34	67.34
1000 R Nf + 2.5 mM MNUA	6.67	24.67	33.34	41.34	48.00	50.67
1500 R Nf + 0.5 mM MNUA	10.00	31.34	42.67	50.67	57.34	58.67
1500 R Nf + 1.0 mM MNUA	8.00	24.67	33.34	48.00	48.67	50.67
1500 R Nf + 2.5 mM MNUA	4.00	18.00	36.67	44.67	46.67	52.00

Table 2. Percent of emergence, growth reduction and survival rate of M_1 soybean plants cv. Warszawska after fast neutron (Nf) and N-nitroso-N-methylurea (MNUA) treatment

Combination of treatment		Percent of reduction		
mutagen	doses	emergence	growth	survival
Control	H_2O	0	0	0
MNUA	0.5 mM	13	-18	43
MNUA	1.5 mM	59	61	45
MNUA	2.5 mM	73	60	80
Nf	500 R	-24	-6	20
Nf	1000 R	-52	-11	25
Nf	1500 R	-20	20	45
Nf +MNUA	500 R +0.5mM	-14	40	19
Nf +MNUA	500 R +1.5 mM	10	63	42
Nf +MNUA	500 R +2.5 mM	31	60	76
Nf +MNUA	1000 R+0.5 mM	-56	20	22
Nf +MNUA	1000 R+1.5 mM	13	34	41
Nf +MNUA	1000 R+2.5 mM	35	40	94
Nf +MNUA	1500 R+0.5 mM	-61	14	38
Nf +MNUA	1500 R+1.5 mM	7	55	69
Nf +MNUA	1500 R+2.5 mM	74	85	90

A very significant difference occurred in the survival percent of plants in different treatment combinations. The use of chemical mutagen caused quite high reduction of survival, and fast neutrons at different doses of irradiation caused a shift towards percentage reduction of plant survival lower by half (Table 2). The combined treatment of Nf with 2.5 mM MNUA lowered the survival of soybean plants up to 90% - it means, that such doses are critical for soybean. In the combinations with lower concentrations of both mutagens some kind of synergistic effect of Nf and MNUA was observed. For example - considering the effect of somatic damage of each separate treatments with 1.5 mM MNUA (45 % reduction of plant survival) and 1000 R irradiation (25% reduction) one could expect the reduction of survival of about 70% in treatment combination: 1000 R Nf + 1.5 mM MNUA. As it is shown from Table 2, reduction in plant survival in this treatment combination was only 40%. So, it was even less than after separate 1.5 mM MNUA treatment.

The results of somatic damage in the M_1 generation were confirmed by field experiments with M_2 generation soybean lines (Table 3). As could be seen from the data, the combined treatment with Nf+ MNUA caused a significant increase of plant survival, nearly doubling the rate of growing plants in relation to the simple MNUA treatment.

Analysing the influence of the combined treatment with Nf and MNUA allowed to evaluate growth stimulation with low doses of radiation observed in soybean plants even in the M_2 generation (Fig. 1).

Table 3. Variability in emergence, percent of survival and early mature plant frequency in the M_2 generation of soybean plants cv. Warszawska, after fast neutron (Nf) and N-nitroso-N-methylurea (MNUA) treatment

Combination of treatment		Number of sown seeds	Emergence			Survival plants		Early mature plants (%)
mutagen	dose		number of plants	%	reduction (% of control)	number	%	
Control	H_2O	600	402	67.0	0.0	229	38.2	-
MNUA	0.5 mM	454	213	45.9	31.5	95	20.5	28.12
MNUA	1.5 mM	419	157	37.4	44.2	48	11.5	39.58
MNUA	2.5 mM	181	53	29.3	56.3	7	3.9	16.67
Nf	500 R	883	478	54.1	19.2	229	25.9	45.87
Nf	1000 R	905	567	62.6	6.5	228	25.2	31.72
Nf	1500 R	308	195	63.3	5.5	55	17.8	5.15
Nf +MNUA	500 R +0.5mM	721	536	74.3	-11.0	160	22.2	48.46
Nf +MNUA	500 R +1.5 mM	456	287	65.9	1.6	104	22.8	1.87
Nf +MNUA	500 R +2.5 mM	190	140	73.7	-10.0	50	26.3	0.00
Nf +MNUA	1000 R+0.5 mM	694	415	59.8	10.7	218	31.4	19.07
Nf +MNUA	1000 R+1.5 mM	413	146	35.3	47.2	49	11.9	4.67
Nf +MNUA	1000 R+2.5 mM	30	10	33.3	50.3	5	16.7	0.00
Nf +MNUA	1500 R+0.5 mM	435	229	52.6	21.4	54	12.4	25.00
Nf +MNUA	1500 R+1.5 mM	105	38	36.2	46.0	9	8.6	11.11
Nf +MNUA	1500 R+2.5 mM	84	28	33.3	50.3	18	21.4	11.11

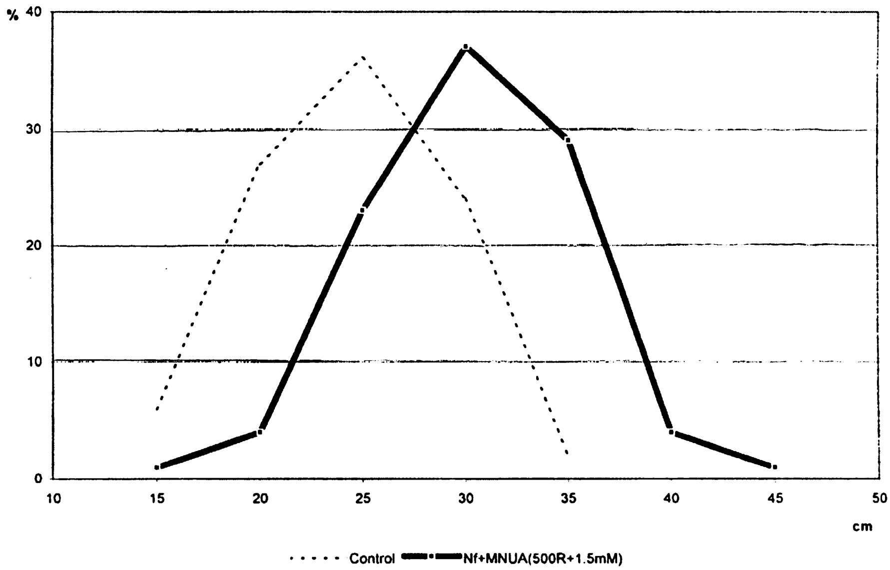

Fig. 1. Stimulation effect of the low doses of Nf irradiation combined with MNUA treatment on plant height in the M_2 generation.

Assessment of the chlorophyll mutants frequency in the M_2 generation - the test commonly used in mutagenesis, in order to determine the level of genetic changes - with respect to soybean was useless. The chlorophyll mutants occurred very seldom.

The frequency of genetic changes in the soybean genome and its relation to the treatment combination was evaluated using the number of plants with earlier maturity in comparison to War- szawska cultivar (Table 3). The number of soybean mutants with earlier ripening obtained with 500 R dose of fast neutron irradiation was higher, than with the highest effective concentration of chemical mutagen (1.0-1.5 mM MNUA).

CONCLUSIONS

- The results showed some kind of protection effect of radiation on the level of somatic damage in soybean plants.

- The phenomenon of the "delaying effect" was noted, because the protection effect created by fast neutron radiation in the combined treatments with chemomutagen was noted in the emergence and plant survival in the M_2 generation as well.

- From the point of view of genetic changes induced in the soybean genome, the most effective dose of fast neutron irradiation was the dose of 500 R. For the soybean cv. Warszawska the optimum dose of mutagens was 500 R Nf, used before MNUA treatment with the concentrations in the range from 1.0 to 1.5 mM.

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