

Mutational Efficiency of Thermal Neutrons in *Lens esculenta* (MOENCH)

J. UHLÍK

University of Agriculture, Department of Genetics and Plant Breeding, Praha*

Received August 3, 1970

Abstract. First findings on the mutation efficiency of thermal neutrons in lentil are presented.

- 1) The highest proportion of segregating progenies with chlorophyll mutants and chimeric plants reached 22% when doses of 1.6×10^{12} n cm⁻² and 2×10^{12} n cm⁻² were used.
- 2) After irradiation with doses in the range from 2×10^{12} n cm⁻² to 2.6×10^{12} n cm⁻² different chlorophyll mutants were produced at the same time in 2 to 4% of the offsprings.
- 3) The maximum proportion of the progenies in which only the viridis mutants were segregated was 18%, that with xanthoviridis mutants alone 6%, with xantha 4%, with albina alone 2%, with chimeric plants alone 8%.
- 4) The highest number of various types of mutants together with various combinations of two types of mutants in the same progeny were induced by doses of 2×10^{12} n cm⁻² and 2.3×10^{12} n cm⁻².
- 5) The highest number of induced chlorophyll mutants per 1 offspring was 0.44 mutants after irradiation with the dose of 2.3×10^{12} n cm⁻².
- 6) The highest number of chlorophyll mutants per 100 of planted seeds was 1.46 mutants after a dose of 3×10^{12} n cm⁻².

From published papers summarizing the effects of neutron irradiation of plants (GOPAL-AYENGAR and SWAMINATHAN 1963, SMITH 1961, 1969, UHLÍK 1970) it may be concluded that due to the relatively small number of studies there is so far no general agreement as to the biological efficiency of thermal neutrons and especially to some specific phenomena which they produce. For a number of cultivated plants which offer the possibility of mutational improvement there are either no data on their radiosensitivity to neutrons or the data are not easy to compare.

The present paper summarizes the first findings concerning the mutational efficiency of thermal neutrons in lentil.

* Address: Praha 6-Suchbát, Czechoslovakia.

Material and Methods

The study of mutational efficiency was carried out in offspring of irradiated plants of lentil (*Lens esculenta* MOENCH) cv. "Hrotovecká veľkozrnná", cultivated in 1967 in a season very suitable for cultivation, in southern Slovakia at the Plant Breeding Station of Topoľníky. For the irradiation of seeds 9 doses of thermal neutrons were used, ranging from $6.6 \times 10^{11} \text{ n cm}^{-2}$ to $3.3 \times 10^{12} \text{ n cm}^{-2}$. The irradiation of the material was carried out in the atomic pile of the Institute of Nuclear Research of the ČSAV, at Řež near Prague. The output of the pile was 2 MW, the neutron flux amounting to $10^9 \text{ n cm}^{-2} \text{ s}^{-1}$.

From fifty randomly chosen plants of the M_1 generation all seeds capable of germination were planted, irrespective of their numbers. The seeds were planted in soil in a greenhouse in areas of $10 \text{ cm} \times 2 \text{ cm}$. The temperature in the greenhouse was between 15° and 23° C .

The state of plants was evaluated from the 12th to the 18th day after planting. The mutational efficiency was estimated from the relative numbers of chlorophyll mutants and chimeric plants. In the offspring, chlorophyll mutants viridis, xanthoviridis, xantha and albina were quantitatively followed. Chimeric plants included seedlings with isolated light-green, yellow or white parts of leaves and stems, or with leaves markedly spotted.

When following the chlorophyll mutants the proportion of segregating progenies with one mutant or with more types of mutants in the same progeny, proportions of individual progeny with a given type of mutant, as well as the number of mutants per progeny and per 100 planted seeds of plants of the M_1 generation were evaluated.

Results

A) Proportion of Segregating Progenies in the Total Number of Analyzed Progenies

The highest proportion of segregating progenies (22%) was produced by doses of $1.6 \times 10^{12} \text{ n cm}^{-2}$ and $2 \times 10^{12} \text{ n cm}^{-2}$ (Table 1). With both decreasing and increasing doses the proportion of segregating progenies was regularly reduced.

Irradiation with lower doses produced in individual progenies only chlorophyll mutants of a single type. Only after irradiation with doses of $2 \times 10^{12} \text{ n cm}^{-2}$, $2.3 \times 10^{12} \text{ n cm}^{-2}$ and $2.6 \times 10^{12} \text{ n cm}^{-2}$ two types of different chlorophyll mutants were produced in the same offspring in 2 to 4% of the offspring. When applying the highest doses again only chlorophyll mutants of a single type were segregated.

If the efficiency of individual doses of irradiation is estimated solely from the proportion of the progenies segregating only one type of chlorophyll mutants, the dose of $1.6 \times 10^{12} \text{ n cm}^{-2}$, producing mutants and chimeric plants in 22% of the progenies, appears to be most effective.

From the total number of analyzed progenies the relatively highest proportion of progenies (18%) was segregated as viridis mutants, after a dose of $1.6 \times 10^{12} \text{ n cm}^{-2}$ (Table 2, values % S_x). After irradiation with other doses the proportion of these progenies did not exceed 8%. Higher pro-

TABLE 1

TOTAL PROPORTION OF SEGREGATING PROGENIES and proportions of segregating progenies with one or more types of mutants in the same progeny

Dose [n cm ⁻²]	Number of progenies			Number of segregating progenies with mutants			
				of 1 type		of more types	
	<i>S^x</i>	<i>S^o</i>	% <i>S^x</i>	<i>S</i>	% <i>S^x</i>	<i>S</i>	% <i>S^x</i>
Control	50	0					
6.6 × 10 ¹¹	50	5	10	5	10		
10 ¹²	50	4	8	4	8		
1.3 × 10 ¹²	50	7	14	7	14		
1.6 × 10 ¹²	50	11	22	11	22		
2.0 × 10 ¹²	50	11	22	9	18	2	4
2.3 × 10 ¹²	50	9	18	7	14	2	4
2.6 × 10 ¹²	50	6	12	5	10	1	2
3.0 × 10 ¹²	50	5	10	5	10		
3.3 × 10 ¹²	50	5	10	5	10		

S^x — total number of analyzed progenies

S^o — total number of segregating progenies

S — number of segregating progenies with mutants of one or more types in the same progeny

% *S^x* — proportion of definite segregating progenies from total number of analyzed progenies

portions of progenies with the viridis mutants only were found after irradiation with relatively low doses.

The proportion of the progenies with xanthoviridis mutants only reached the maximum value of only 6%. A relatively high proportion of these offspring was produced by medium doses of radiation.

With the progenies segregating xantha mutants only relatively high proportions were found after relatively high doses. The maximum proportion of these progenies found was 4%.

The progenies with albina mutants alone were found only after irradiation with a dose of 3 × 10¹² cm⁻² in 2% of all progenies.

The maximum proportion found with the chimeric plants only was 8%. The relative frequency of these progenies was found to increase regularly with the radiation dose and to reach the above maximum when medium doses were used.

B) Proportions of the Individual Segregating Progenies in the Total Number of all Segregating Progenies

From the whole population of all segregating progenies those with viridis mutants alone were again the most frequent (Table 2, values % *S^o*). The highest proportions (8.2% and 8.0%) were found after irradiation with doses of 1.6 × 10¹² n cm⁻² and 3.3 × 10¹² n cm⁻². The dependence of the number of progenies on the magnitude of the dose exhibits two maxima.

The maximum proportion of progenies with xanthoviridis mutants was produced only with the relatively low dose of 6.6 × 10¹¹ n cm⁻². In progenies

TABLE 2

PROPORTIONS OF SEGREGATING PROGENIES IN THE TOTAL NUMBER OF ANALYZED PROGENIES AND PROPORTIONS OF SEGREGATING PROGENIES WITH GIVEN TYPE OR COMBINATION OF CHLOROPHYLL MUTATIONS

Dose [n cm ⁻²]	Progenies		Progenies segregating														
			only mutants										at the same time mutants				
	S ^x	S ⁰	I'			XV			X			A			Ch		
			S	% S ^x	% S ⁰	S	% S ^x	% S ⁰	S	% S ^x	% S ⁰	S	% S ^x	% S ⁰	S	% S ^x	% S ⁰
Control	50	0															
6.6 × 10 ¹¹	50	5	2	4.0	40.0	2	4.0	40.0	1	2.0	20.0						
50	50	4	2	4.0	50.0												
1.3 × 10 ¹²	50	7	5	10.0	71.5	1	2.0	14.2	1	2.0	14.2						
1.6 × 10 ¹²	50	11	9	18.0	82.0				1	2.0	9.0						
2.0 × 10 ¹²	50	11	1	2.0	9.1	3	6.0	27.3	2	4.0	18.2						
2.3 × 10 ¹²	50	9	1	2.0	11.1	2	4.0	22.2									
2.6 × 10 ¹²	50	6	1	2.0	16.7				2	4.0	33.4						
3.0 × 10 ¹²	50	5	2	4.0	40.0	1	2.0	20.0	1	2.0	20.0	1	2.0	20.0			
3.3 × 10 ¹²	50	5	4	8.0	80.0												

S^x — number of analyzed progenies

S⁰ — number of segregating progenies

S — number of progenies segregating either mutants of a definite type alone, or a definite combination of two types of mutants in the same progeny

% S^x — per cent of segregating progenies from the total number of analyzed progenies

% S⁰ — per cent of definite segregating progenies from the total number of segregating progenies

Progenies with mutants: V — viridis, XV — xanthoviridis, X — xantha, A — albina, Ch — chimeric plants.

Combination in progeny — XV, V

— Ch, V

with xantha mutants only higher frequencies were found after relatively high radiation, whereas in those with chimeric plants only the high frequencies were caused by medium doses.

The same holds for progenies in which two types of chlorophyll mutants were segregated at the same time. Such progenies were found when doses from the narrow range 2×10^{12} n cm⁻² to 2.6×10^{12} n cm⁻² were used. The proportion of progenies in which some of the examined types of chlorophyll mutants were segregated and that of their combinations in the total number of segregating progenies produced by individual doses is given also in Table 2.

The highest number of various types of mutants together with the combinations of two different types of mutants were produced by doses of 2×10^{12} n cm⁻² and 2.3×10^{12} n cm⁻². After irradiation with these doses the proportion of progenies segregating viridis mutants was pronouncedly reduced.

When doses higher or lower than those from 2×10^{12} n cm⁻² to 2.3×10^{12} n cm⁻² were used the number of different types of mutants as well as of their combinations was decreased, representing from 2 to 4 types (Table 5).

TABLE 3

TOTAL NUMBER OF MUTANTS per 100 planted seeds of plants of M₁ generation and per progeny

Dose [n cm ⁻²]	Number of seeds	Number of progenies	Number of mutants		
			S	per 100 seeds	per progeny
Control	4 588	50	0		
6.6×10^{11}	4 662	50	7	0.148	0.14
10^{12}	4 369	50	7	0.159	0.14
1.3×10^{12}	3 481	50	7	0.199	0.14
1.6×10^{12}	2 523	50	15	0.593	0.30
2.0×10^{12}	2 353	50	20	0.852	0.40
2.3×10^{12}	2 567	50	22	0.858	0.44
2.6×10^{12}	1 892	50	19	1.000	0.38
3.0×10^{12}	1 239	50	18	1.460	0.36
3.3×10^{12}	1 383	50	12	0.867	0.24

S — the total number of chlorophyll mutants produced with the given dose

C) Number of Mutants per Analyzed Progeny

The highest number of chlorophyll mutants produced per progeny was 0.44. If the maximum values found are considered, the highest proportion per progeny was that of the viridis mutants (0.22), the frequencies of other mutants decreasing in the sequence xanthoviridis (0.20), chimeric plants (0.12), xantha (0.10), albina (0.04). The values corresponding to individual doses are given in Table 4.

D) Number of mutants per 100 seeds

The highest number of chlorophyll mutants per 100 seeds was 1.46 after irradiation with 3×10^{12} n cm⁻². The maximum number of mutants of

a definite type per 100 seeds was found to decrease in the sequence viridis (0.79), xanthoviridis (0.52), xantha (0.32), chimeric plants (0.23) and albina (0.16). The values corresponding to individual doses are given in Table 4.

Discussion

In agreement with the majority of studies evaluating the mutational efficiency of thermal neutrons in various cultivated plants (*cf.* UHLÍK 1970) it was found that irradiation of lentil with thermal neutrons also produces effects which can be used for mutational plant breeding. The composite radiation of the Czechoslovak atomic pile, which includes thermal neutrons as a substantial component, may be used for practical purposes of mutational plant improvement as demonstrated by sufficiently high proportions of both segregating progenies and mutants.

A characteristic effect of thermal neutrons may be seen in the production of a relatively high proportion of segregating progenies with chimeric plants. On the other hand, a relatively low number of xantha mutants was produced as compared with the frequency of other mutants of rare occurrence in lentil, especially with that of the albina mutant.

When comparing the mutational efficiency of the composite radiation of the atomic pile of the Institute of Nuc-

TABLE 4

NUMBER OF INDIVIDUAL TYPES OF MUTANTS PER 100 PLANTED SEEDS OF M_1 GENERATION AND PER PROGENY

Dose [n cm ⁻²]	Num- ber of seeds	Num- ber of pro- genies	V			XV			X			A			Ch		
			S	per 100 seeds	per prog.	S	per 100 seeds	per prog.	S	per 100 seeds	per prog.	S	per 100 seeds	per prog.	S	per 100 seeds	per prog.
Control	4 588	50	0	0.04	0.04	0	0.08	0.08	0	0.02	0.02	0			0		
6.6×10^{11}	4 662	50	2	0.04	0.04	4	0.08	0.08	1	0.02	0.02	1			1	0.02	0.02
10^{12}	4 369	50	2	0.04	0.04	4	0.09	0.08									
1.3×10^{12}	3 481	50	5	0.14	0.10	1	0.28	0.02	1	0.02	0.02						
1.6×10^{12}	2 523	50	10	0.39	0.20				3	0.11	0.06				2	0.07	0.04
2.0×10^{12}	2 353	50	3	0.12	0.06	9	0.38	0.18	5	0.21	0.10				3	0.12	0.06
2.3×10^{12}	2 567	50	9	0.35	0.18	7	0.27	0.14							6	0.23	0.12
2.6×10^{12}	1 892	50	4	0.21	0.08	10	0.52	0.20	3	0.15	0.06				2	0.10	0.04
3.0×10^{12}	1 239	50	8	0.64	0.16	4	0.32	0.08	4	0.32	0.08	2	0.16	0.04			
3.3×10^{12}	1 383	50	11	0.79	0.22										1	0.07	0.02

S — total number of chlorophyll mutants of given type produced with the given dose
Mutants: V — viridis, XV — xanthoviridis, X — xantha, A — albina, Ch — chimeric plants

TABLE 5

NUMBERS OF VARIOUS TYPES OF CHLOROPHYLL MUTANTS and their various combinations in progenies

Dose [$n\text{ cm}^{-2}$]									
$10^{11} \times$	$10^{12} \times$								
6.6	1.0	1.3	1.6	2.0	2.3	2.6	3.0	3.3	Control
Numbers of different types of chlorophyll mutants:									
3	2	3	3	4	3	3	4	2	0
Number of various combinations of different mutants in the same progeny:									
0	0	0	0	1	2	1	0	0	0
Number of different types of chlorophyll mutants and their combinations in progenies:									
3	2	3	3	5	5	4	4	2	0

lear Research of the Czechoslovak Academy of Sciences with the results obtained by other authors with various species of cultivated plants it must be kept in mind that the radiosensitivity of various experimental plants, various effects of factors capable of influencing the radiosensitivity of the irradiated material, the mode of evaluation, as well as the composition of radiation produced by different atomic piles, may vary rather substantially from case to case.

References

- GOPAL-AYENGAR, A. R., SWAMINATHAN, M. S.: Use of neutron irradiation in agriculture and applied genetics. — In: Biological Effects of Neutron and Proton Irradiation, IAEA Vienna I: 409—432, 1963.
- SMITH, H. H.: Neutron irradiation biological effects. — Science **143**: 1466—1472, 1961.
- SMITH, H. H.: Neutron irradiation of seeds as a tool in plant genetics and breeding. — Jap. J. Genet. **44** (suppl.): 443—453, 1969.
- UHLÍK, J.: Biologické účinky neutronového záření na kulturní rostliny. [Biological effects of neutron irradiation on crop plants.] — Studijní Informace ÚVTI (Praha), Základní věda v zemědělství **1970** (1), 1970.

J. UHLÍK, Vysoká škola zemědělská v Praze, katedra genetiky a šlechtění: **Mutační účinnost tepelných neutronů u *Lens esculenta* (MOENCH).** — Biol. Plant. **13**: 216—223, 1971.

V práci jsou uvedeny první poznatky o mutační účinnosti tepelných neutronů u čočky. Účelem práce bylo i zjištění dosud netestovaného biologického účinku neutronů produkovaných atomovým reaktorem Ústavu jaderného výzkumu ČSAV.

1. Nejvyšší podíl štěpících potomstev s chlorofylovými mutanty a chimerickými rostlinami činil 22 % po užití dávek $1,6 \times 10^{12} \text{ n cm}^{-2}$ a $2 \times 10^{12} \text{ n cm}^{-2}$.

2. Po ozáření dávkami v rozmezí $2 \times 10^{12} \text{ n cm}^{-2}$ až $2,6 \times 10^{12} \text{ n cm}^{-2}$ vznikli u 2 % až 4 % potomstev současně rozdílní chlorofyloví mutanti.

3. Potomstev, v nichž vyštěpili pouze mutanti viridis bylo maximálně 18 %, potomstev pouze s mutanty xantoviridis 6 %, s xanta 4 %, jen s albina 2 %, jen s chimerickými rostlinami 8 %.
4. Nejvíce různých druhů mutantů spolu s rozdílnými kombinacemi mutantů dvou druhů v témž potomstvu indukovaly dávky 2×10^{12} n cm⁻² a $2,3 \times 10^{12}$ n cm⁻².
5. Největší počet indukovaných chlorofylových mutantů připadajících na 1 potomstvo činil 0,44 mutanta po ozáření dozí $2,3 \times 10^{12}$ n cm⁻².
6. Největší počet chlorofylových mutantů připadajících na 100 vyšetých semen činil 1,46 mutanta po ozáření dávkou 3×10^{12} n cm⁻².