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INDUCTION OF ARTIFICIAL MUTATIONS IN *LINUM USITATISSIMUM* L. BY EXPOSING SEEDS TO DIFFERENT DOSES OF *X*-RAYS

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Abstract: *The artificial induction of mutations represents an effective method for generating new sources of germplasm, which are essential in plant breeding. In the present study, induced mutagenesis was applied to flax by exposure to X-rays. As biological material, three flax (Linum usitatissimum L.) genotypes were selected: Kaufmann and Blaringhem (oilseed flax), and Belinka (fiber flax). Seeds were irradiated with X-rays at three doses: 500, 700, and 900 Gy, using the RS-2400-Q installation. The treatment led to a significant reduction in plant viability, especially for the Blaringhem and Belinka genotypes, which were completely eliminated within the first 24–33 days. In the case of the Kaufmann genotype, the survival rate at the 500 Gy dose was only 0.7%, also indicating high sensitivity to irradiation. However, plant mortality during various developmental stages was also influenced by environmental stress factors (hydrological drought, high temperatures).*

Analysis of the M_0 generation descendants revealed significant morphological changes compared to the control: increased number of branches, flowers, and fruits, but a pronounced reduction in seed mass and quality. The M_0 descendants exhibited an imbalance between vegetative and reproductive growth.

Keywords: flax, linseed, Linum usitatissimum L., physical mutagenesis, X rays.

INTRODUCTION

Flax or linseed (*Linum usitatissimum* L., 2n = 2x = 30) - technical, oilseed, medicinal, melliferous, nutritional and ornamental plant. It is an annual, dicotyledonous, self-pollinated plant species, with a relatively small genome size, estimated at ~370-455 Mb, depending on the genotypes [1, 2].

Currently, approximately 61,000 germplasm accessions of linseed including 1,127 wild accessions are conserved in genebanks (1,750 - GB) and/or institutes worldwide [3, 4]. However, estimates show that only 10,000-15,000 of them are unique [5, 6]. Including 12 mutant flax varieties developed through induced mutagenesis, all of

which have been officially approved and registered in the MVD of FAO/IAEA.

According to the FAO/IAEA Mutant Varieties Database (MVD), the total number of officially recorded mutant varieties currently stands at 3,460, derived from over 200 to 240 plant species [7, 8, 9, 10, 11, 12, 13]. This figure reflects a significant increase since the 1990s, with the number of registered varieties rising from 1,936 in 1990 to 2,724 in 2000, 3,222 in 2010, and 3,397 by 2020 [7].

The MVD database indicates that the species with the highest number of mutant varieties include rice (over 876 entries), barley (309), chrysanthemum (288), wheat (276), soybean (184) and maize (89) etc. In the case of flax, 12 mutant varieties have been registered, corresponding to two different species: *Linum usitatissimum* L. (6 – textile flax, 5 – oilseed flax) and *Linum grandiflorum* Desf. (1 – red flax, decorative). The countries with the most mutant crops: China (825), Japan (501), India (346), Russian Federation (216), Netherlands (176) etc. [13, 14].

The earliest flax mutant variety recorded in the Mutant Variety Database (MVD) is *"Redwood 65"*, an oilseed flax cultivar officially registered in Canada in 1965. This variety was developed through *X*-ray-induced mutagenesis, a method involving physical mutagens. The most recent mutant oilseed flax variety, *"DLV 20"*, was registered in 2019 in India and was obtained via ethyl methanesulfonate (EMS)-induced mutagenesis, a chemical mutagenesis technique. In the case of textile flax, the most recently recorded mutant varieties are *"Baltyuchai"* and *"M-5"*, both registered in the Russian Federation in 1991. These were developed using chemical mutagens, specifically nitrosoethylurea (NEU) and dimethyl sulfate (DMS). Additionally, the ornamental flax variety *"Zorepad"*, belonging to the species *Linum grandiflorum* Desf., was developed in Ukraine through EMS-induced chemical mutagenesis and was officially registered in 2003 [7].

Physical mutagens, mostly ionizing radiation, can increase the natural mutation rate by 1,000 to 1 million-fold, and have been widely used to induce heritable genetic changes. More than 70% of induced and released mutant crop varieties have been developed using physical mutagens. Since the 1960s, gamma rays have become the most commonly used mutagenic agent in plant mutation breeding [15].

MATERIALS AND METHODS

The scientific research was conducted at the Institute of Genetics, Physiology, and Plant Protection of Moldova State University, as well as at the Slovak University of Agriculture in Nitra, Institute of Plant and Environmental Sciences.

For the research, three flax genotypes from the *ex situ* collection of the Gene Bank were used: *Kaufmann* and *Blaringhem* (oil flax), and *Belinka* (fiber flax).

The flax seeds was subjected to *X*-ray irradiation treatment using the RS-2400-Q installation, equipped with an ionizing *X*-ray emitter (Rad Source

Technologies). The irradiation was performed in a single session, using 1000 seeds for each experimental variant. Three radiation doses were applied: 500 Gy (with an exposure time of 44 minutes), 700 Gy (62 minutes) and 900 Gy (80 minutes). During the treatment, the seeds were placed in cylindrical carbon fiber canister. The control samples consisted of untreated flax seeds, used for comparison with the irradiated variants.

The assessment of biological, morphological, and agrotechnical parameters was conducted in accordance with the criteria established in the *Descriptors List for Flax (Linum usitatissimum L.)*, Nitra, 2016 [16]. The evaluations were carried out following the technical standards and methodological guidelines officially approved for plant breeding activities [17, 18].

RESULTS AND DISCUSSIONS

For obtaining induced mutations in plants, physical mutagens are used (gamma rays, *X*-rays and ultraviolet (*UV*) radiation). These methods allow the induction of heritable changes in the plant genome, which can then be selected based on desired traits (disease resistance, productivity, fruit size etc.).

The effects of ionizing radiation on plants vary significantly. The ultimate impact of the mutagenic agent, including *X*-rays, on plants is contingent upon the administered dose, the nature of the mutagen, the botanical species involved and the susceptibility of the genotype under investigation.

During the growing season, a significant elimination of the irradiated material was recorded. Under the influence of radiation and limiting environmental factors, the *Blaringhem* (after 24 days) and *Belinka* (after 33 days) varieties proved to be the least resistant, with the irradiated plants being completely eliminated. Also, the percentage of remaining plants was very low for the *Kaufmann* variety as well, reaching only 0.7% (500 Gy) (Table 1).

The analysis of the data presented in Table 2 highlights significant differences between the descendants from the M_0 variant (irradiated plants) and *Kaufmann* (control). In the case of M_0 descendants, the technical stem length could not be determined, as these plants developed basal branches, unlike the control plants, in which branching predominantly occurred in the upper part of the stem. A specific characteristic of the irradiated forms is the higher number of branches, flowers, and fruits produced. However, these plants show a considerable reduction in the number of viable seeds, seed mass, and seed quality. Thus, in the M_0 variant, although a double number of main branches was recorded (14.14) and flowering was abundant (~98.14 flowers/plant), only 45.0 of these developed into fruits, with a total seed mass of only 0.93 g. In comparison, the control plants showed an average of 7.14 branches, 32.0 flowers, and 26.9 capsules (fruits), associated with a correspondingly higher seed mass.

Table 1. Determination of the degree of elimination of plants under the influence of *X*-rays

Day	Dose of ionising radiation, Gy	Genotype			Day	Genotype		
		Kaufmann	Belinka	Blaringham		Kaufmann	Belinka	Blaringham
d. 3	500	65.0%	77.5%	54.6%	**d. 27**	4.7%	3.8%	0
	700	51.0%	55.1%	52.5%		0.3%	0.1%	0
	900	72.7%	50.0%	24.4%		0.4%	0	0
	Martor	100%	100%	100%		50%	50%	50%
d. 6	500	60.0%	57.5%	43.4%	**d. 29**	2.5%	1.0%	0
	700	31.7%	27.1%	39.2%		0.1%	0.1%	0
	900	30.3%	20.0%	11.1%		0.1%	0	0
	Martor	100%	100%	100%		50%	50%	50%
d. 9	500	48.6%	44.3%	24.1%	**d. 31**	1.9%	0.4%	0
	700^{+}	28.8%	25.9%	27.1%		0.1%	**0**$^{+}$	0
	900*	25.7%	24.4%	66.0%		**0***	0	0
	Martor	100%	100%	100%		50%	50%	50%
d. 12	**500**$^{+}$	41.4%	40.5%	17.5%	**d. 33**	1.3%	**0**$^{+}$	0
	700*	21.6%	19.2%	13.3%		**0***	0	0
	900	19.2%	66.0%	0.5%		0	0	0
	Martor	100%	100%	100%		50%	50%	50%
d. 16	500	34.3%	29.7%	3.0%	**d. 37**	1.1%	0	0
	700	15.0%	12.0%	2.0%		0	0	0
	900^{-}	10.0%	15.0%	**0**$^{-}$		0	0	0
	Martor	90%	90%	90%		50%	50%	50%
d. 19	**500**$^{-}$	21.7%	21.3%	**0**$^{-}$	**d. 38**	10	0	0
	700	6.7%	1.6%	0.3%		0	0	0
	900	3.5%	0.5%	0		0	0	0
	Martor	75%%	75%%	75%		50%	50%	50%
d. 24	500	9.0%	7.0%	0	**d. 40**	0.9%	0	0
	700^{-}	0.7%	0.3%	**0**$^{-}$		0	0	0
	900	0.6%	0.1%	0		0	0	0
	Martor	75%	75%	75%		50%	50%	50%
d. 26	500	5.4%	5.0%	0	**d. 125**	0.7%	0	0
	700	0.4%	0.1%	0		0	0	0
	900^{+}	0.5%	**0**$^{+}$	0		0	0	0
	Martor	50%	50%	50%		49%	49%	49%

The ratio between the number of flowers and the number of fruits formed is 53.14 in the M_0 variant, compared to only 5.1 in the control variant, indicating low reproductive efficiency in the irradiated plants, despite their seemingly greater morphological potential.

Table 2. Comparative analysis of linear and quantitative parameters of the *Kaufmann* control with the descendants from generation M_0 (500 Gy)

Parameters per plant	***Kaufmann***	
	Control	**M_0 (500 Gy)**
Plant height, cm	45±1,20	41±1,63
Technical stem length, cm	26±1,36	0
No. of branches, un.	7,14±0,4	14,14±1,47
No. of capsules, un.	26,9±3,62	45,0±10,44
No. of flowers, un.	32,0±3,72	98,14±10,19
No. of seeds per plant, un	7,14±0,74	5,71±0,57
Mass of seeds per plant, g	1,28±0,12	0,93±0,34

CONCLUSIONS

The M_0 generation plants, subjected to *X*-ray radiation treatment, exhibited heightened sensitivity under simultaneous exposure to abiotic stress factors such as hydrological drought and high temperatures, which prevailed during the vegetation period. The cumulative interaction of these factors led to a significant increase in the physiological vulnerability of the plants, as they were unable to develop effective tolerance mechanisms to ionizing radiation in the context of combined environmental stress.

The study aimed to evaluate the response of several genotypes (*Kaufmann*, *Blaringhem*, *Belinka*) to the combined action of physical mutagenic factors (*X*-ray irradiation) and environmental conditions. The results highlighted that the *Kaufmann* genotype exhibited the highest resistance under the applied experimental conditions, demonstrating superior tolerance to the induced stress. Regarding the effectiveness of the irradiation doses, the 500 Gy treatment proved to be the most effective in stimulating mutagenic processes. These findings suggest that the *Kaufmann* genotype and the 500 Gy dose may represent an optimal combination for generating genetic variability in breeding programs.

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