

EXPRESSION OF BREEDING-VALUABLE TRAITS IN AMARANTH LINES OBTAINED BY INDUCED MUTAGENESIS

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Topicality. In order to meet international standards, the gene pool of amaranth lines and varieties with high seed yields, improved grain and seed quality characteristics, and an early maturity should be expanded. To accelerate the breeding process in amaranth, experimental mutagenesis plays a crucial role as a modern breeding method that enhances genetic variability, providing breeders with new initial material for selection and further variety development. Most of these varieties have been obtained using physical mutagens, primarily gamma radiation. **Purpose.** To assess the seed quality characteristics of new mutant amaranth lines in terms of protein, oil content, and fatty acid composition compared to the original varieties. **Materials and Methods.** Three varieties of white-seeded amaranth (*Amaranthus hypochondriacus* L.) Studentskyi, Kharkivskyi 1, and Sem developed by the V. V. Dokuchaev Kharkiv National Agrarian University (now – the State Biotechnology University) were evaluated for seed quality characteristics. Additionally, seven mutant lines LMST15, LMS150ChR, LMS150ChN, LMH150, LMH150RV, LMS150ZV, and LMS150ChN induced by gamma irradiation were evaluated. The analysis of oil content, protein content, and fatty acid composition of amaranth seeds was performed at the Laboratory of Genetics, Biotechnology, and Quality of the Yuriev Plant Production Institute of the National Academy of Agrarian Sciences of Ukraine. Statistical methods were used to determine the reliability of the results. **Results.** The conducted research demonstrated that gamma irradiation is a powerful factor capable of significantly altering the characteristics of amaranth plants. The obtained amaranth lines with modified qualitative traits represent valuable initial material for breeding. These lines exhibited increased protein content (by 1.56–3.35 %), enhanced oil content in seeds (by 0.43–0.76 %), and increased behenic, linoleic, and oleic acid content (by 0.10 %, 1.2–2.0 %, and 6.3 %, respectively). **Conclusions.** Based on the study, mutant breeding-valuable lines LMH150 (IU072495), LMS150ChR (IU072494), LMS150ChN (IU072492), and LMST15 (IU072493) characterised by a set of improved grain quality traits were identified. This confirms the feasibility of using radiation mutagenesis in amaranth breeding to obtain mutants with modified valuable economic traits, thereby expanding the range of initial breeding material for this crop.

Key words: amaranth, gamma irradiation, mutant, protein, oil, fatty acid composition

Introduction. Amaranth is a high-protein ancient agricultural crop of Southern and south-western part of North America, where it was cultivated by the Maya, Aztec and Inca civilisations. Interest in amaranth cultivation increased at the end of the 20th century when the first detailed biochemical studies of the amaranth grain were conducted [1, 2]. Among the representatives of the *Amaranthaceae* family, particular attention is drawn to grain amaranth species, which include *Amaranthus hypochondriacus* L., whose food and market potential has not yet been realised [3, 4]. According to UN experts and other scientists, amaranth is considered the most promising grain crop of the 21st century [5]. Due to its special biochemical composition, amaranth seeds are higher in protein content (16–18 %) than crops such as wheat (12–14 %),

maize (9–10 %), barley (12–13 %), as well as in lysine and tryptophan content [6, 7]. In terms of nutritional value, amaranth is a natural mixture of rice and beans, as the average protein and amino acid content in amaranth grains is identical to that of major cereals and legumes [8]. According to various scientific studies, the lipid composition of amaranth grain varies considerably depending on the species, genotype and growing conditions, ranging from 1.9 % to 9.7 %. The main part of the fatty acids in amaranth oil includes linoleic (47 %), oleic (26 %), palmitic (19 %), and linolenic (1.4 %) acids of their total content [9]. Amaranth oil also has a high content of unsaturated hydrocarbon squalene, which accounts for 2.4–8.0 % of the essential extract, depending on the species [10, 11].

The European amaranth market, including

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Ukraine, has grown significantly over the last 30 years of cultivation. First of all, this is due to the fairly large percentage of people who choose organic food or vegetarianism in general. According to forecasts, the European amaranth market will grow annually by an average of 10.8% until 2028. However, amaranth remains a niche crop in the EU and Ukraine today. Therefore, the production of amaranth products depends on imports from American and Asian countries [12]. In particular, amaranth cultivation is actively developing in the United Kingdom, France, Italy, Germany, Lithuania, Ukraine and other European countries [7, 13, 14]. Ukraine's domestic needs for amaranth products are covered by its own production. The area sown with grain amaranth in Ukraine is about 4,000 ha.

Among the breeding methods for improving fatty acid composition, protein and lipid content, there are various methods of induced mutagenesis, including the use of gamma irradiation, as presented in the studies by Kpocheme et al. [15]. According to the conclusions of scientific publications, the radiation mutagenesis is effective in improving the biochemical composition of amaranth grain [16–18].

Amaranth is a relatively new crop in Ukraine, with insufficiently studied lipid and protein composition indicators, and new amaranth breeding forms obtained as a result of induced mutagenesis have not been studied at all. Therefore, the research was aimed at determining the quality indicators of seeds of new mutant amaranth lines in terms of protein and oil content and fatty acid composition compared to the original varieties.

Materials and Methods. During the period 2022–2023, field trials were conducted at the experimental field of the Genetics, Plant Breeding and Seed Production Department of the State Biotechnology University (at the Dokuchaievsk village) to develop new source material of amaranth of the species *A. hypochondriacus* L. using induced mutagenesis (gamma rays). Three amaranth varieties, such as Kharkivskiy 1, Sem, and Studentskiy, selected by the V. V. Dokuchaiev Kharkiv National Agrarian University (now the State Biotechnological University), and seven mutant lines, such as LMST15, LMSt150ChR, LMSt150ChN, fat extracted is calculated as the difference be-

LMKh150, LMKh150RV, LMS150ZV, LMS150ChN, induced by gamma irradiation (irradiation source – ^{60}Co . Treatment location – National Scientific Centre Institute of Metrology (Ukraine, Kharkiv). Installation – DETU 12-05-02).

Sowing, crop management, phenological observations and records were carried out in accordance with the Methodology for State Variety Testing of Agricultural Crops [19].

The study was conducted in the eastern part of the Left-Bank Forest-Steppe of Ukraine. Weather conditions varied during the years of the study, thus contributing to the determination of the response of amaranth varieties and mutant lines to their variability.

In 2022, favourable weather conditions prevailed during the spring period, characterised by sufficient precipitation (43.4 mm), with an average long-term indicator of 43.7 mm. The average air temperature (18.4 °C) exceeded the long-term average by 2.2 °C, which had a positive effect on the early stages of development of the samples under study. The conditions in spring 2023 were characterised by cooler weather compared to the long-term average, with an average temperature in May of 13.5 °C, compared to the long-term average of 15.1 °C. At the same time, there was more precipitation in May (108.3 mm) compared to the long-term average (48 mm). The summer period was generally characterised by higher average daily temperatures.

Chemical analysis of amaranth seeds for oil and protein content and fatty acid composition was carried out in the Laboratory of Genetics, Biotechnology and Quality at the Yuriev Plant Production Institute of NAAS of Ukraine. The protein content was determined using the Kjeldahl method, in accordance with DSTU ISO 5983-1 (2005), selecting an average sample weighing 5 g for analysis.

The oil content was determined using S. V. Rushkovsky's gravimetric method with the Soxhlet apparatus. The method is based on the principle of determining the fat content by the defatted residue. Samples of dried and ground seeds are placed in filter paper bags (50 × 70 mm in size and weighing about 250 mg each) and extracted with diethyl ether in a Soxhlet apparatus until completely defatted. The average seed sample for analysis was 3 g. The amount of between the initial weight and the weight of the

defatted residue.

The fat content (x) in the sample taken was calculated using the formula:

$$x = \frac{(a - b)(c - d)}{(a - b)} \times 100$$

where: a – mass of air-dry bag with absolutely dry sample, mg; b – mass of air-dry bag, mg; c – mass of absolutely dry bag with absolutely dry defatted sample, mg; d – mass of absolutely dry bag, mg.

The fatty acid composition of oil from amaranth mutant seeds was determined using a SelmiChrom chromatograph by gas-liquid chromatography.

Results and Discussion. After analysing the variability of biochemical indicators in the seeds of mutant samples selected from amaranth varieties, it was established that gamma irradiation causes the induction of amaranth lines that

differ from the control in terms of protein content in seeds. In particular, the following mutant lines induced by a dose of 150 Gy were obtained from the Studentskyi variety: LMSt150ChR – 18.0 %, LMSt150ChN – 17.2 % (compared to 15.4 % in the control). From the Kharkivskyi 1 variety, the mutant line LMH150 was selected (at a dose of 150 Gy), which was characterised by a protein content in seeds of 19.9 %, which is 3.4 percentage points higher than the control (16.6 %). In the control variant of the Sem variety, the protein content in the seeds was 17.17 %, while in the mutant sample of the LMS150ChN it was 18.68 %, i.e. the sample under study exceeded the control by 1.51 percentage points.

In terms of oil content in seeds, almost all mutant lines were equal to the control variants (Table 1).

Table 1. Protein and oil content in seeds of amaranth varieties and ma mutant lines

Variety, mutant line	Protein content, %			Oil content, %		
	2022	2023	average	2022	2023	average
Studentskyi (control)	15.39	15.43	15.41	7.33	7.29	7.31
LMST15	16.30	16.34	16.32	7.75	7.81	7.78
LMSt150ChR	18.00	18.10	18.05	7.13	7.05	7.09
LMSt150ChN	17.14	17.20	17.17	6.00	5.94	5.97
<i>LSD</i> ₀₅	-	-	0.50	-	-	0.45
Kharkivskyi 1 (control)	16.55	16.63	16.59	7.44	7.50	7.47
LMKh150	19.90	19.98	19.94	5.40	5.30	5.35
LMKh150RV	16.61	16.65	16.63	7.46	7.40	7.43
<i>LSD</i> ₀₅	-	-	0.43	-	-	0.50
Sem (control)	17.15	17.19	17.17	6.77	6.81	6.79
LMS150ChN	18.65	18.71	18.68	6.22	6.28	6.25
LMS150ZV	16.70	16.66	16.68	7.60	7.54	7.57
<i>LSD</i> ₀₅	-	-	0.49	-	-	0.48

The exceptions were lines with reduced oil content: LMSt150ChN – 5.97 % (7.31 % – in the control) and LMKh150 – 5.35 % (7.47 % – in the control). In addition, the mutant LMS150ZV (Sem variety) induced by a dose of 150 Gy was selected, which exceeded the control (6.79 %) by 0.78 percentage points for the studied trait.

Based on the results of the analysis of the fatty acid composition of amaranth mutant lines, lines with an increased content of certain acids were selected. In the Student mutant sample LMS15, induced by a radiation dose of 15 Gy, the seeds contained 35.88 % linoleic acid, compared to 33.70 % in the control. The mutant LMSt150ChN had an elevated content of stearic

(4.45%), linoleic (35.45 %), linolenic (1.12 %) and behenic (0.46 %) acids compared to the control (3.67, 33.70, 0.90 and 0.28 %, respectively). The mutant line LMSt150ChR was characterised by the content of linoleic (38.20 %), linolenic (1.13 %) and behenic (0.43 %) acids (compared to the control values of 33.70, 0.90 and 0.28 %, respectively). The mutant LMKh150 (in the Kharkivskyi 1 variety at 150 Gy) had an elevated content of oleic (42.15 %), linolenic (1.22 %) behenic (0.40 %) acids compared to the control (35.87, 0.93, 0.30 %, respectively) (Table 2). As a result of the study, mutants with altered qualitative characteristics (with increased content of oil and behenic, linoleic and pal-

Table 2. Fatty acid composition in seeds of mutant amaranth plants

Variety, mutant line	Composition										
	Myristic acid	Palmitic acid	Palmitoleic acid	Unidentified component	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosanoic (arachidonic) acid	Eicosenoic acid	Behenic acid
Studentskyi (control)	0.14	19.93	0.50	0.38	3.67	39.40	33.70	0.90	0.90	0.20	0.28
	0.15	20.04	0.55	0.36	3.61	39.23	33.77	0.90	0.87	0.25	0.27
LMST15	0.15	20.48	0.55	0.33	3.53	36.98	35.88	0.86	0.78	0.24	0.22
	0.15	20.60	0.50	0.34	3.56	37.05	35.80	0.90	0.72	0.18	0.20
LMSt150ChR	0.13	19.93	0.44	0.55	3.64	34.80	38.20	1.00	0.85	0.12	0.34
	0.15	20.21	0.40	0.54	3.57	34.67	38.07	1.13	0.85	0.18	0.23
LMSt150ChN	0.20	18.89	0.40	0.67	4.38	37.37	35.38	1.07	1.02	0.16	0.46
	0.23	18.80	0.47	0.70	4.45	37.25	35.45	1.12	1.04	0.20	0.29
Kharkivskyi 1 (control)	0.18	21.50	0.51	0.51	3.48	35.87	35.64	0.93	0.94	0.14	0.30
	0.19	21.55	0.50	0.45	3.42	35.70	35.80	1.04	0.87	0.20	0.28
LMKh150	0.18	20.20	0.44	0.63	3.80	42.15	29.76	1.22	1.00	0.22	0.40
	0.17	20.40	0.48	0.65	3.70	42.23	29.92	1.08	0.94	0.16	0.27
LMKh150RV	0.16	21.34	0.45	0.37	3.45	36.10	35.86	0.92	0.87	0.16	0.32
	0.17	21.30	0.54	0.30	3.53	36.24	35.72	0.98	0.83	0.18	0.21
Sem (control)	0.21	22.20	0.52	0.53	2.90	33.63	37.60	1.20	0.77	0.19	0.25
	0.19	22.34	0.46	0.55	2.95	33.70	37.40	1.15	0.78	0.18	0.30
LSM150ChN	0.23	21.07	0.55	0.57	3.77	25.75	45.62	1.05	0.98	0.11	0.30
	0.20	21.30	0.55	0.58	3.82	25.70	45.39	1.00	0.92	0.12	0.42
LMS150ZV	0.17	21.15	0.48	0.37	3.30	37.83	34.41	0.95	0.76	0.22	0.36
	0.18	21.10	0.46	0.49	3.50	37.92	34.00	0.94	0.91	0.23	0.27

mitoleic acids) were identified as valuable source material for breeding.

In particular, a collection of morphological mutants with altered quantitative and qualitative characteristics has been formed.

Based on the research conducted, the following mutant amaranth lines were selected as valuable for breeding: LMS15 (Studentskyi variety), induced by a radiation dose of 15 Gy, which exceeded the control in terms of protein content by 0.9 %, palmitoleic acid by 0.11 %, stearic acid by 0.32 %, arachidic acid by 0.15 % and behenic acid by 0.20 %; mutant LMSt150ChN, obtained under the action of a dose of 150 Gy, exceeded the control variant in protein content by 1.76 %, stearic acid – by 0.78%, linoleic acid – by 1.75%, linolenic acid – by 0.22% and behenic acid – by 0.18%; the mutant sample LMSt150ChR, induced by a gamma irradiation dose of 150 Gy, exceeded the control in protein content by 2.64 %, linoleic acid by

4.5 %, linolenic acid by 0.23 % and behenic acid by 0.15 %. From the Kharkivskyi 1 variety, a mutant line LMKh150 was selected, which was induced by a radiation dose of 150 Gy and exceeded the control in terms of protein content in seeds (by 3.35%) and fatty acid composition as follows: oleic acid (by 6.28 %), linolenic acid (by 0.29 %), and behenic acid (by 0.10 %). The mutant with pink tips in the panicle (dose of 150 Gy) was characterised by an increased content of oil (by 0.79 %), linoleic acid (by 2.62 %) and eicosenoic acid (by 0.13 %). A sample with pink panicles and black seeds was obtained from the Sem variety, induced by a radiation dose of 150 Gy, which exceeded the control in terms of protein content (by 1.93 %), stearic acid (by 0.92 %), linoleic acid (by 8.02 %) and arachidic acid (by 0.20 %).

Mutant lines LMKh150 (IU072495), LMSt150ChR (IU072494), LMSt150ChN (IU072492), LMST15 (IU072493) were selec-

ted and transferred to the National Centre for Plant Genetic Resources of Ukraine for implementation in the breeding process..

Our findings are confirmed by the results of studies conducted by other researchers. In 2008, scientists I. Gajdosova and E. Palencharova conducted research at the Biochemistry and Biotechnology Department of the Slovak University of Agriculture in Nitra (Slovak Republic), which showed an increase in the protein content of amaranth seeds as a result of gamma irradiation [7, 17]. They found that gamma irradiation at a dose of 175 Gy on *A. cruentus* and hybrid K-33 (*A. hybridus* x *A. hypochondriacus*) promotes the development of mutants with increased protein content compared to the original forms (by 1.1–1.5 %).

Conclusions. According to the results of

the studies, we have found that:

- lines with altered seed quality characteristics – with increased protein content (by 1.56–3.35 %), oil in grain (by 0.43–0.76 %), with increased content of behenic, linoleic, oleic acids (by 0.10 %, 1.2–2 and 6.3 %, respectively) are valuable source material for amaranth breeding.

- radiation mutagenesis in amaranth is useful for obtaining mutants with altered parameters of valuable economic traits in order to extend the range of source material in the amaranth breeding.

- four mutant lines LMKh150 (IU072495), LMSSt150ChR (IU072494), LMSSt150ChN (IU072492), LMST15 (IU072493), which were developed and transferred to the National Centre for Plant Genetic Resources of Ukraine for use in the breeding process.

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Актуальність роботи полягає в необхідності розширення генофонду ліній та сортів амаранту, які відповідають світовим стандартам – високоврожайних за насінням, поліпшених за якісними показниками зерна та насіння, скоростиглих. Для прискорення селекційного процесу в амаранту велике значення має використання експериментального мутагенезу, який є одним із сучасних методів селекції, що дає змогу збагачувати ресурси за генетичною мінливістю, даючи селекціонерам новий вихідний матеріал для проведення добору і в подальшому – для створення сортів, більшість яких одержані при застосуванні фізичних мутагенів (в основному гамма-променів). **Метою досліджень** було оцінити якісні показники насіння, нових мутантних ліній амаранту за вмістом білка, олії та жирнокислотного складу, порівняно з вихідними сортами. **Матеріали і методи.** Оцінку показників якості насіння визначали у трьох сортів амаранту білонасінного (виду *Amaranthus hypochondriacus* L.) Студентський, Харківський І і Сем селекції Харківського національного аграрного університету ім. В. В. Докучаєва (зараз – Державний біотехнологічний університет) та семи мутантних ліній ЛМСТ15, ЛМСт150ЧР, ЛМСт150ЧН, ЛМХ150, ЛМХ150РВ, ЛМС150ЗВ, ЛМС150Ч, індукованих гамма-опроміненням. Аналіз насіння амаранту за вмістом олії, білка та жирнокислотного складу робили в лабораторії генетики, біотехнології та якості Інституту рослинництва ім. В. Я. Юр'єва НААН України. Визначення достовірності результатів проводили статистичним методом. **Результати** досліджень показали, що гамма-опромінення відносяться до потужних чинників, здатних суттєво змінювати ознаки рослин амаранту. Отримані лінії зі зміненими ознаками якості становлять цінний вихідний матеріал для селекції: з підвищеним вмістом білка (на 1,56–3,35 %), олії в зерні (на 0,43–0,76 %), з підвищеним вмістом бегенової, лінолевої, олеїнової кислот (на 0,10 %, 1,2–2 та 6,3 % відповідно). **Висновки.** На основі проведених досліджень виділено мутантні селекційно-цінні лінії (ЛМХ150 (IU072495), ЛМСт150ЧР (IU072494), ЛМСт150ЧН (IU072492), ЛМСТ15 (IU072493), які характеризуються підвищеним комплексом показників якості зерна. Це свідчить про доцільність використання радіаційного мутагенезу у амаранту з метою отримання мутантів зі зміненими параметрами корисних господарських ознак, необхідних для розширення спектра вихідного матеріалу в селекції цієї культури.

Ключові слова: амарант, гамма-опромінення, мутант, білок, олія, жирнокислотний склад