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Abstract: The article presents research results of the γ-ray and fast neutrons impact on various rice species, in order to obtain mutant forms resistant to salinity (NaCl) and drought factors (sorbitol. They are going to be used as initial forms in synthetic selection when creating varieties, adapted to the stressful conditions of, both, soil and climate in the Kazakhstan Aral Sea. The average lethal doses (LD50) of γ-rays and fast neutrons, as well as, the average NaCl and sorbitol lethal concentrations were established. Suchenvironment is best suited for clear manifestation of the mutagenic effect of ionizing radiation and the resistance of rice plants to salinity and drought. A distinct dependence in the effect of ionizing radiation and stress factors on the number of induced resistant mutant forms has been related to the initial rice sort. The largest number of mutant forms was obtained from the local variety Syr Suluy, followed by Leader and AyKerim varieties. From all varieties, the number of mutants obtained from exposure to γ-rays was 43 pieces out of 4500 grains, and from the impact of fast neutrons - 115 pieces out of 2700 grains. M1 mutant plants significantly differ from the initial forms in terms of morphological features – plant height, panicle length, grain size. Most plants are characterized by short growth and even dwarfism (<80 cm). They are lodging tolerant, have short and highly sterile panicles, indicating that they are mutants and resistant to salinity, drought, or both.

Keywords: rice; variety; selection; mutagenesis; gamma rays; fast neutrons; salinity; drought tolerance

Introduction

The Kyzylorda region is the main rice-growing region of the Republic of Kazakhstan. Located on the territory of the Kazakhstan Aral Sea region Kyzylord is not spared by intensive desertification, salinization and soil deflation [1]. This harsh conditions for the creation of salt-and drought-resistant rice varieties that are characterized by high productivity, initial growth intensity, diseases and pests resistance, as well as, and high grain quality [2,3].

At the same time, there is a tendency to reduce the varietal diversity of rice, which significantly increases their genetic vulnerability. This occurrence is based on a genetic uniformity increase of rice species. Therefore, the source material is of decisive importance and requires constant renewal, also, introduction of new economically valuable genes and their complexes into it. In order to achieve the set goals, one of the effective breeding methods is induced mutagenesis, which is considered all over the world as a source of creating fundamentally new forms. That would facilitate the expansion of

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the synthetic breeding possibilities through the use of mutant forms in the hybridization process that have unique breeding and valuable traits. In this regard, when breeding new rice varieties adapted to the stressful soil and climatic conditions of the Kazakhstan Aral Sea region, a significant role is played by radiation breeding. This technique makes possible the obtaining of mutant lines resistant to abiotic stress factors, as well, as lines with individual or a complex of positive traits [4–6].

In recent years, scientists from various countries have begun to intensively use induced mutagenesis [7, 8], including the use of gamma rays and the use of salinity and drought factors [9], in order to create new forms of mutant lines resistant to stress factors. Therefore, since 2021, we have begun research on the effect of γ-rays and fast neutrons on rice plants combined with salinity (NaCl) and drought (sorbitol) on seeds of different varieties [10–14].

Materials and methods

The following certified methods were used in the presented work:

1. Guidelines for the study of the world collection of rice and the classifier of the genus Oriza L [15].

2. Methodology of the field experiment [16].

3. Methodology for conducting variety testing of agricultural plants [17].

Research is carried out in 2 stages: Stage 1 - selection of rice varieties and treatment of their seeds with various doses of γ-rays and fast neutrons, as well as solutions of sodium chloride (0.5-1%) and sorbitol (3.75-7.5%), in order to determine the optimal doses (LD50) of mutagens and the optimal salinity and drought concentrations; Stage 2 - selection of promising mutant lines resistant to salinity and drought, as well as their further study in laboratory and field conditions for the heritability of acquired traits and their breeding value.

As experimental materials were used the rice seeds of the AiKerim, Lider and Syr Suluy sorts. They are approved for use and widespread in the Kyzylorda region of the Republic of Kazakhstan.

To determine the average lethal dose, the seeds of the Syr Suluy variety were irradiated with 5 doses of γ-rays (50, 100, 150, 200 and 250 Gy), then the seeds of 3 varieties were irradiated with an average lethal dose (LD50) using an ILU-electronic accelerator. 10 JSC "Park of Nuclear Technologies" (Kurchatov, Republic of Kazakhstan) in the following process parameters:

- radiation energy, E - 4 MeV;

-current value, I cf. (this is the current of electrons on the target, it is desirable to indicate the gamma - producing a nuclear reaction) - 6.84 mA;

- range of absorbed doses - 100 Gy.

To determine the average lethal dose, the seeds of the Syr Suluy variety were irradiated with 5 doses of fast neutrons (5, 25, 50, 75 and 100 Gy), then the seeds of 3 varieties were irradiated with an average lethal dose (LD50) using the EG-5 electrostatic generator from the Frank Laboratory of Neutron Physics, part of the Joint Institute for Nuclear Research (Dubna, RF) with the fallowing parameters:

- generation of neutrons with energy E - 4.1 MeV;
- deuteron beam current (reaction D(d,n)3He) - 1.7-2.0 μA, energy - 2.5 MeV;
- neutron flux intensity - 3×107 particles/cm2;
- average number of particles passing through the sample - 35-40 million particles/hour;

The radio sensitivity of seeds was determined on the basis of growth indicators at the initial stage of ontogeny - the energy of germination and laboratory germination of seeds, the height and weight of 15-day-old seedlings.

To establish the sensitivity to salinity and drought factors, unirradiated seeds of the Syr Suluy variety were left to germinate in a thermostat. For NaCl aqueous solution concentrations were: 0; 0.5; 1.0; 1.5; 2.0 and 2.5%, as well as 0.5; 10; 15 and 20% of sorbitol.

The sort sensitivity to salinity and drought and their average lethal concentration (LD50) were defined through the energy of growth and seed germination, as well as, the height and weight of the 15-day-old seedlings.

Seeds of three varieties, irradiated with medium lethal doses (LD50) of γ-rays and fast neutrons, were treated with average lethal concentrations of NaCl and sorbitol. Also, the irradiated seeds were treated with half the LD50 concentration of each of the stress factors together.

Results and discussion

As a result of the research, it was discovered that the average lethal dose (LD50) of γ-rays for rice is 100 Gy, and for fast neutrons - 50 Gy. The average lethal concentration of NaCl is 1.0% aqueous solution, and for sorbitol - 7.5% [9].

In terms of seed germination, the largest numbers of germinating grains in all varieties were obtained from NaCl variants with when, both irradiated with γ-rays (21.0-62.6%) and with fast neutrons (47.0-72.0%). The smallest number of germinating grains appeared with the sorbitol treatment (Table 1).

Table 1. - Laboratory germination and survival of seeds treated with γ-rays, fast neutrons and salinity (NaCl) and drought factors (sorbitol), 2022.

Variety name	Type and dose of irradiation	Impact factors and their optimal concentrations, %	Number of treated seeds, pcs.	Number of seedlings		Survivors before transplanting plants	
				total, pieces	%	total, pieces	%
1	2	3	4	5	6	7	8
AyKerim	γ – rays 100Gr	NaCl – 1,0%	500	226	45,2	3	1,3
		Sorbitol – 7,5%	500	15	5,0	1	6,7
		NaCl+Sorbitol (0,5%+3,75%)	500	18	1,6	1	5,6
	Fast neutrons, 50 Gr	NaCl – 1,0%	300	168	56,0	6	3,4
		Sorbitol – 7,5%	300	13	4,3	2	15,4
		NaCl+Sorbitol (0,5%+3,75%)	300	16	5,3	2	12,5
Leader	γ – rays 100 Gr	NaCl – 1,0%	500	319	62,6	0	0,0
		Sorbitol – 7,5%	500	21	2,2	6	28,6
		NaCl+сорбит (0,5%+3,75%)	500	31	6,2	8	25,8
	Fast neutrons, 50Gr	NaCl – 1,0%	300	141	47,0	1	0,7
		Sorbitol– 7,5%	300	105	21,0	12	11,4
		NaCl+Sorbitol (0,5%+3,75%)	300	135	45,0	14	10,4
Sir Suluy	γ – rays 100 Gr	NaCl – 1,0%	500	105	21,0	9	8,6
		Sorbitol – 7,5%	500	36	1,2	3	8,3
		NaCl+Sorbitol (0,5%+3,75%)	500	51	10,2	12	23,5
	Fast neutrons, 50 Gr	NaCl – 1,0%	300	216	72,0	30	13,9
		Sorbitol – 7,5%	300	61	20,3	9	14,8
		NaCl+Sorbitol (0,5%+3,75%)	300	147	49,0	30	20,4

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However, 10 days after the seedlings emergence, despite, the of seedling's watering termination with NaCl and sorbitol solutions, most seedlings began to die, especially gamma-ray irradiated species. As a result, 20 days after seed's germination in various experiment versions, only 1 to 30 plants remained alive. They were transplanted to paddy-fields in special containers, 35× 15 × 15 cm in size and filled with soil taken from the original paddy-fields rice.

At the same time, the largest number of surviving plants (3- 30 pcs.) was observed in the Syr Suluy variety, followed by the Leader variety (1 -14 pcs.) and the smallest number turned out to be for the AiKerim variety (1 - 6 pcs.). According to the number of surviving plants prior to transplantation most distinguishable results were as follows: the AiKerim sort with NaCl (3 and 6 pcs.), the Leader variety with sorbitol (6-12 pcs.) and NaCl + sorbitol (8 and 14 pcs.), the Syr Suluy variety has variants with NaCl (9 – 30 pcs.) and NaCl + sorbitol (12 and 30 pcs.).

Plants that survived gamma rays and fast neutrons treatment, as well as, salinization (NaCl) and drought (sorbitol) factors and placed in the special paddy-field containers during their tillering phase, were further transplanted directly into the soil. During growing season, phenological observations of plant growth and development were carried out, besides, two-time fertilization with nitrogen. Before harvest, the remaining plants were counted. All surviving plants were removed from the root for biometric analysis and photographing.

The results of the phenological observations revealed that almost all altered forms had interphase periods prolongation and retardation in the growth and development of the plants, except for the individual mutant plants of the cultivar AyKerim (Table 2). The observed very high level of panicle emptiness and low plant growth confirm the manifestation of mutational processes under the influence of ionizing radiation.

Table 2. Duration of interphase periods and vegetation period of M1 plants obtained after treatment of rice seeds with gamma rays, fast neutrons and salinization factors (NaCl) and drought (sorbitol), 2022.

Name of the variety	Type of irradiation	Salinization and drought factor	Duration of the interphase period, days				Growing season, days
			flooding of seedlings	shoots - tillering	tillering - earing	earing – full ripeness	
1	2	3	4	5	6	7	8
AiKerim Leader	γ – rays	NaCl	13-14	33-35	31-32	43-45	119-125
		Sorbitol	13	33	32	43	121
		NaCl+ Sorbitol	13	33	32	44	122
	Fast neutrons	NaCl	12-13	32-34	33-35	43-46	120-128
		Sorbitol	13	32	35-36	44	124-125
		NaCl+ Sorbitol	12-13	31-32	36-37	43-45	122-127
	Control	-	12	32	30	42	116
Syr Suluy	γ – rays	NaCl	No shoots				
		Sorbitol	12-13	33-35	31-33	44-46	120-127

		NaCl+ Sorbitol	12-13	32-35	31-33	44-45	119-126
	Fast neutrons	NaCl	12	34	33	45	124
		Sorbitol	12-13	32-33	31-33	44-16	119-125
		NaCl+ Sorbitol	12-13	33-34	31-33	44-46	120-126
	Control	-	12	32	31	43	118
	γ – rays	NaCl	11	32	30	41-42	114-115
		сорбит	11	33	30-31	42-43	116-118
		NaCl+ сорбит	12	33	31-32	42-45	117-122
	Fast neutrons	NaCl	10-12	31-33	31-34	41-44	113-123
		сорбит	10-11	32-33	32-34	42-45	116-123
		NaCl+ сорбит	11-12	31-33	31-35	43-46	116-126
	Control	-	10	30	28	37	105

Thus, when compared to control species under the same conditions (influence of ionizing radiation and salinization and drought) the extension of interphase periods resulted in the elongation of vegetation period for: the AiKerim variety by 3-12 days, in the Leader variety by 1-9 days and in the Syr Suluy variety by 9-21 days. It should be noted that all transplanted M1 plants, which were described and subjected to biometric analysis, were completely preserved at harvest.

The biometric analysis results of mutant lines resistant to salinization and drought indicated that, depending on the variety, irradiation type and stress factor, obtained mutants sharply differ, both from the original forms and among themselves in quantitative indicators of various traits (Table 3).

Table 3. – Biometric characteristics of mutant M1 lines obtained after irradiation with ionizing radiation and treatment with salinization and drought factors, 2022.

Name of the mutant	Plant height, cm.	Bushiness, pcs.		The main panicle					Grain weight per plant, g.
		General	Productive	Length, cm.	Number of grains, pcs.		Emptiness,%	Grain weight, g.	
					full	puny			
1	2	3	4	5	6	7	8	9	10
AiKerim variety									
Original form	121	4,4	4,4	19,6	107	42	28,2	3,17	11,79

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M_1A-1-1-3	113-126	4-6	1	21,0-24,0	28-76	87-118	53,4-80,8	1,00-2,58	1,80-4,75
M_1A-1-2-1	98,0	16	6	22,0	142	7	4,7	4,69	21,69
M_1A-1-3-1	112,0	11	3	25,0	66	102	60,7	2,31	4,33
M_1A-2-1-7	113-127	3-13	1-4	21,0-25,5	38-156	23-130	12,8-74,3	1,23-5,11	1,71-18,26
M_1A-2-2-2	112-115	13-16	1-3	20,0-26,0	85-116	24-126	17,1-59,7	3,00-3,85	4,68-16,31
M_1A-2-3-2	109-110	14-17	1-5	20,5-23,0	73-90	72-129	44,4-63,9	2,15-3,21	2,38-14,20
Leader variety									
Original form	94	7,0	7,0	16,6	156	42	21,2	4,14	28,88
M_1L-1-1-0	Absent								
M_1:L-1-2-6	69-83	5-13	1-2	11,0-14,5	5-84	24-84	22,2-92,1	0,11-1,96	0,11-4,59
M_1L-1-3-8	62-78	3-13	0-1	11,0-13,5	0-48	43-90	61,3-100,0	0,00-1,19	0,00-2,57
M_1L-2-1-1	91,0	47	25	16,0	104	65	38,5	3,20	20,32
M_1L-2-2-13	55-78,5	2-14	0-3	10,5-15,0	0-81	22-105	24,4-100,0	0,00-2,26	0,00-3,88
M_1L-2-3-16	53-79	1-19	0-4	11,0-16,0	0-98	31-91	24,6-100,0	0,00-2,45	0,00-5,70
Syr Suluy variety									
Original form	88	5,2	5,2	18,0	86	35	28,9	2,84	13,25
M_1C-1-1-9	47-71	2-8	0-4	7,0-13,0	0-31	2-56	6,9-100,0	0,00-1,08	0,00-3,29
M_1C-1-2-3	56-62	12-13	0-2	12,5-15	0-18	30-75	62,5-100,0	0,00-0,55	0,00-0,75
M_1C-1-3-12	49-73	4-10	0-4	6,5-13,5	0-33	3-62	10,0-75,0	0,00-0,99	0,00-2,77
M_1C-2-1-30	48-67	3-12	0-4	8,0-14,0	0-40	4-51	16,6-	0,00-	0,00-3,39

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							100,0	1,29	
M_1C-2-2-14	47-72	3-22	0-4	9,0-14,0	0-67	2-96	2,9-100,0	0,00-2,29	0,00-5,82
M_1C-2-3-30	51-72	1-14	0-3	8,0-19,0	0-32	2-57	7,4-100,0	0,00-2,07	0,00-5,00

Where, M_1 is a mutant of the first generation;
A - Aikerim variety, C – Syr Suluy variety, L- leader variety;
The first digit: 1 – γ- rays; 2 – fast neutrons.
The second digit: 1 - NaCl; 2 – Sorbitol; 3 – NaCl + Sorbitol.
The third digit is the number of mutant plants.

A significant number of resistant to stress factors mutants were obtained from Syr Suluy variety (98 PCs.), then come the Leader (44 PCs.) and Aikerim (16 PCs.) sorts. The number of mutants originating from the γ- rays: the Aikerim variety-10 pcs., the Leader variety -14 pcs., the Syr Suluy variety - 24 pcs., total - 43 pcs. The number of mutants after the fast neutrons impact amount to to 11; 30; 74 pcs, respectively., in total - 115 pcs.

Of these, resistant to NaCl are: from the Aykerim sort - 10 pcs., Leader sort - 1 pcs.and Syr Suluy - 30 pcs. Resistant to sorbitol: Aykerim variety - 3 pcs., in the Leader variety - 19 pcs. and the variety Syr Suluy - 17 pcs. Resistant to NaCl + sorbitol: in the variety Aykerim - 3 pcs., in the variety Lider - 24 pcs., in the variety Syr Suluy - 42 pcs. There is a significant difference between the mutants and the initial species in quantitative terms. Thus, the height of altered plants of the AiKerim variety actually changed in both directions, increase of up to 127 cm and decrease of up to 98 cm. Whereas the Leader and Syr Suluy sorts, mutants were 11-41 cm lower than the originals (Figures 1–3).

AiKerim variety　　Leader variety　　Syr Suluy variety

Figure 1.

Large fluctuation amplitude of indicators is observed in general and productive tillering for the mutant species. The total productivity of mutants, therefore, ranged from 1 to 47 pcs., and 4.4 – 7.0 pcs. for the initial plants. At the same time, the mutants had very low rates of productive tillering (0 - 4 pcs.), compared to the original species (4.4 – 7.0 pcs.).

The main panicles of the altered AiKerim plants appeared to be 0.4-6.4 cm longer than the original form (19.6 cm). The shapes of the Leader and Syr Suluy varieties were 0.6 - 6.1 cm and 3.0 – 11.5 cm, respectively, shorter than the initials.

In 2022, a very high percentage of grain desolation was also observed in the initial forms (21.2-28.9%). This was largely due to frequent interruptions in the supply of irrigation water during the flowering of rice plants, especially mutant plants, which contributed to an acute grain fertility decrease.

A similar pattern is observed for the weight of grain mass from all panicles and from a single plant.

According to the research results of the radiation mutagenesis method using selected factors such as salinity and drought, the obtained mutant forms are resistant to the stressful conditions of the Kazakhstan region of the Aral Sea. They are going to be used as initial material in synthetic breeding, as well as, in the cultivation of new varieties by direct propagation of altered species. Those species are with a complex of economically valuable traits, including stress-resistant, characteristics.

Conclusions

It has been established during the irradiation of rice varieties like AjKerim, Lider and Syr Suluy that the average lethal dose of y-rays is 100 Gr, and for fast neutrons is 50 Gr. The average lethal concentration for these varieties were NaCl - 1,0 %; sorbitol – 7,5 %.

The highest values of germination rates in all varieties were obtained on the variants: NaCl + gamma rays (21.0-62.6%) and NaCl + fast neutrons (47.0-72.0%). However, further, most of the seedlings died on the 20th day and in various variants there were only from 1 to 30 plants that were transplanted into specially flooded rice checks. These plants were completely preserved until the end of harvesting.

In the isolated plants, an extension of the interphase and vegetation periods from 3 to 21 days was observed, depending on the variety, type of irradiation and the factor of salinity and drought.

The largest number of mutant lines resistant to stress factors were obtained in the Syr Suluy variety (98 pcs.), in the Leader varieties (44 pcs.) and AjKerim (16 pcs.). The number of mutants received from exposure of gamma-rays was 10 pcs in the AjKerim variety, 14 pcs in the Leader variety and 24 pcs in the Syr Suluy variety. (a total of 43 pcs.), and from the effects of fast neutrons, respectively, amounted to - 11; 30; 74 pcs., (a total of 115 pcs.).

All the isolated M1 plants differ significantly from the original forms in morphological features – plant height, length and laceration of panicles. The most plants are characterized by stunting and dwarfism (40-80 cm), as well as shortness and high empty-grain (up to 100%) of panicles, which indicates that they are mutant forms that are resistant to salinity, drought or both stress factors.

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References

1. Umirzakov S.I., Innovative way of development of rice growing in Kazakhstan: problems and prospects // Materials of International. scientific and practical conf. "Scientific and innovative foundations for the development of rice farming in Kazakhstan and foreign countries" Kyzylorda. 2012.- pp.17-20.
2. Podolskikh A.N., Baybosynova S.M., Mamonov L.K., Usenbekov B.N. On the characteristics of the biodiversity of the source material for rice breeding in Kazakhstan // Collection of articles "Scientific foundations and practice of rice growing in Kazakhstan". – Almaty. - 2012. –pp.213-230.
3. Bakirov K. Using the method of induced mutagenesis on rice in order to obtain practically valuable forms// Autoref. For the degree of Candidate of Agricultural Sciences, Almalybak, 1979.P.-21.
4. Bakirov K., Vereshchagin G.A. The use of mutagenic factors in the creation of new varieties of rice. // Bulletin of Agricultural Sciences of Kazakhstan, No. 12, Alma-Ata. 1980. pp.-20-26.
5. Bakiruly K., Tokhetova L.A., Yershin Z.R., Kasymzhanov M.T. The effect of ionizing radiation on the growth processes of rice and barley plants using the electron accelerator of JSC "Nuclear Technology Park" Bulletin of the National Research Center of RK., Issue 1(65), Kurchatov. March.2016.
6. Bakiruly K., Yershin Z.R., Aitzhanov A., Abdyvalieva K.S. Studying the effect of radiation treatment of rice seeds with heavy ions on growth processes in the initial stages of ontogenesis using the DC-60 ion accelerator.//Proceedings of the international scientific and practical conference "Science, Production, Business: Current state and ways of innovative development of the agricultural sector on the example of the Agricultural Holding "Bayserke-Agro" dedicated to the 70th anniversary of the Honored Worker of the Republic of Kazakhstan Dosmukhambetov T.M., Almaty, Volume No. 2, 2019., pp.260-262.

7. V.E. Viana, C. Pegoraro, C. Busanello, A. Costa de Oliveira. Mutagenesis in Rice: The Basis for Breeding a New Super Plant, 2019. DOI:10.3389/fpls.2019.01326 Corpus ID: 207958102.
8. Yanjing Su, Guoqi Zhao, Zhenwu Wei, Changjie Yan and Sujiao Liu //Mutation of Cellulose Synthase Gene Improves the Nutritive Value of Rice Straw // College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China. | The Asian-Australian Association of Animal Production Societies. No. 6 : 800-805. June 2012.
9. Ana Abdelnour-Esquivel, Jason Pérez-Chaves, Miguel Rojas-Chaves, Walter Vargas. Use of gamma radiation to induce mutations in rice (Oriza sativa L.) and the selection of lines with tolerance to salinity and drought. // Costa Rican Institute of Technology (ITCR). February 2020. In Vitro Cellular & Developmental Biology - Plant. DOI:10.1007/s11627-019-10015-5
10. Bakiruly K., Tautenov I.A., Zhalbyrov A.E. Creation of a source material for rice breeding by treating seeds with ionizing radiation. // Bulletin of the Korkyt Ata Kyzylorda University, Kyzylorda, 2022, No. 3(62). pp. 55-64.
11. A.I. Kruglyak, A.S. Doroshkevich, Yu. Aleksiayenak, N.O. Appazov, K.B. Bakiruly,
12. M. Balasoui, M.N. Mirzayev, A.A. Nabiyev, E. Popov // Application of the accelerator mass spectrometry method to study the mechanisms of radiation mutagenesis of rice crops: the current state of the issue // Condensed matter research at the IBR-2, International Conference, Dubna, April 25-29, 2022, 65-66.
13. Kruglyak A.I., Alekseenok Yu.V., Doroshkevich A.S., Appazov N.O., Bakiruly K., Mezentseva Zh.V., Ilyina M.N. Obtaining a drought-resistant variety of rice culture as a result of mutagenesis induced by neutrons generated at the EG-5 installation at JINR // Collection of reports, 1 International Conference "Genetic and radiation technologies in agriculture". October 18-21, 2022, Obninsk, FGBNU VNIIRAE. pp. 142-144.
14. Aleksandr Doroshkevich, Tatuana Zelenyak, Anastasia Kruglyak, Yulia Aleksiavenak, Bozena Jasinska, Afag Madadzada, Maria Balasoui Petre Baddic, Marius Stef, Tran Van Phuc, Le Hang khriong - Phan Luong Tuan, Ivan Ristice, Vesna Teofilovie, Roman Balvanovich, Anca Stanculeseu . Diana Mardare, Carmen Mita, Dorin Luca, Vitaliy Ksenevich, Nurbol Appazov, Kurmambek Bakiruly. The study of the cosmogenic radiation effects on condensed matter and living organisms on the Earth using the EG-5 accelerator (JINR) // Book of Abstract for: The 16th International Symposium on Origin of Matter and Evolution of Galaxies, 25-28 October 2022. Hanoi. Vietnam. – P. 7-8.
15. Anastasiya Kruglyak, Aleksandr Doroshkevich, Yulia Aleksiaenak, Nurbol Appazov, Kurmanbek Bakiruly, Vasilisa Volgina, Ekaterina Didenko, Nelya Dorosukevil, Le Hong Khiêm, Phan Luong Thanh, Tran Van Phuc Mung Mudadzada. Influence of cosmogenic neutron radiation on the evolution of terrestrial biological forms on the example of rice and Oyster mushrooms cultures // Book of Abstract for: The 16th International Symposium on Origin of Matter and Evolution of Galaxies, 25-28 October 2022. Hanoi. Vietnam. – 35 p.
16. Methodological guidelines for the study of the world collection of rice and the classifier of the genus Oriza L, Leningrad, VIR, 1974. – 25 p.
17. Dospekhov B.A. Methodology of field experience. – M., 1985.
18. Methodology of variety testing of agricultural plants. – Astana – 2010.