Creation of salinity and drought-resistant mutant rice forms by ionizing radiation (gamma and neutron radiation)

Kurmanbek Bakiruly[1], Aidos Zhalbyrov[1], Anastasiya Kruglyak[2], Yulia Aleksiayenak[2], Gulsim Baimbetova[1], Zeinolla Yershin[3], Yury Gledenov[2], Aleksander Doroshkevich[2] and Nurbol Appazov[4]

[1] Kazakh Research Institute of Rice named after I. Zhakhaev, Kyzylorda, Kazakhstan
[2]Joint Institute for Nuclear Research, Dubna, Moscow Region, Russia
[3] Joint-stock company «Park of Nuclear Technologies», Kurchatov, Kazakhstan
[4]Korkyt Ata Kyzylorda University, Kyzylorda, Kazakhstan
* Correspondence: 79197709640@yandex.ru

Abstract: The article presents the 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 (sorbite). 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 sorbite lethal concentrations were established. A distinct dependence on the effect of ionizing radiation and stress factors on the number of induced resistant mutant forms has been related to the initial rice variety. The largest number of mutant forms was obtained from the local variety Syr Suluy, followed by Leader and Aikerim varieties. Out of all varieties, only 1% of the initially irradiated by γ-rays seeds survived, while after fast neutron irradiation – 4,3%. M1 mutant plants significantly differ from the initial forms in terms of morphological features – plant height, panicle length, and grain size. They are tolerant to lodging, and have short and highly sterile panicles, indicating that they are mutants.

Keywords: rice; variety; selection; mutagenesis; gamma-rays; fast neutrons; salinity; drought tolerance; LD_{50}

1. 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 Kyzylorda is not spared by intensive desertification, salinization and soil deflation [1–3]. Such harsh conditions are conducive to the creation of salt-and drought-resistant rice varieties that are characterized by high productivity, initial growth intensity, diseases and pest resistance, as well as, and high grain quality [4–8].

At the same time, there is a tendency to reduce the varietal diversity of rice, which significantly increases its genetic vulnerability. This occurrence is based on a genetic uniformity increase in rice species. Therefore, the source material is of decisive importance and requires constant renewal, also, the 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 [9, 10]. That would facilitate the expansion of the synthetic breeding possibilities through the use of mutant forms in the hybridization process that have unique breeding and valuable traits. According to the Food and Agriculture Organization of the United Nations/International Atomic Energy Agency – Mutant Variety Database (FAO/ IAEA-MVD) data reports in 2022 on the developed and officially released mutants 40% of the cereal mutant species are composed of rice mutants [11]. In this regard, when breeding new rice varieties adapted to the stressful soil and cli-

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 April 2023 doi:10.20944/preprints202304.0590.v1

matic 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 [12–15].

In recent years, scientists from various countries have begun to use intensively induced mutagenesis [16, 17], including the use of gamma rays [18, 19] heavy ions [20, 21] and the use of salinity and drought factors [22], in order to create new forms of mutant lines resistant to stress factors. While gamma rays are the most used physical mutagen [17], neutron irradiation of the rice showed good results in the creation of mutants with various characteristics [23–26].

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 (sorbite) on seeds of different varieties [27–31].

2. Materials and Methods

For the study three varieties of rice (the genus *Oriza L*) were selected: Aikerim, Leader and Syr Suluy. They are approved for use and are widespread in the Kyzylorda region of the Republic of Kazakhstan. Seeds of these varieties were subjected to different irradiation: γ-rays and fast neutrons. One of the goals was to determine the median lethal dose (LD_{50}) for these varieties because in the literature there is no unambiguous data for them.

For conducting experiment the following certified guidelines and methodologies were applied:

1. Guidelines for the study of the world collection of rice and the classifier of the genus Oriza L [32].
2. Methodology of the field experiment [33].
3. Methodology for conducting a variety testing for agricultural plants [34].

Study was carried out in two stages: first stage was the selection of rice varieties and their seeds treatment with various doses of γ-rays and fast neutrons, as well as solutions of sodium chloride (0.5-1%) and sorbite (3.75-7.5%), in order to determine the optimal doses (LD_{50}) of mutagens and the optimal salinity and drought concentrations. The second stage was the 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.

To determine the median lethal dose, the seeds of the Syr Suluy variety were irradiated with 5 doses of γ-rays (50, 100, 150, 200 and 250 Gy), and then the seeds of 3 varieties were irradiated with a median lethal dose (LD_{50}). The γ-ray irradiation was conducted at ILU-10 - Electron Linear Accelerator in JSC "Park of Nuclear Technologies" (Kurchatov, Republic of Kazakhstan) with the following parameters:

radiation energy, E, is 4 MeV; the avearage current value, I_{av}, is 6,84 мA. Range of absorbed doses was 200 Gy.

To determine the median 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 a median lethal dose (LD_{50}) 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 following parameters: neutron energy, E, was 4.1 MeV; deuteron beam current (reaction D(d,n)3He) – 1.7-2.0 μA with energy – 2.5 MeV; neutron flux intensity was 3×10^7 particles/cm^2.

The radio sensitivity of seeds was determined based on 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 sorbite.

The variety sensitivity to salinity and drought and their median lethal concentration (LD_{50}) 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 median lethal doses (LD_{50}) of γ-rays and fast neutrons, were treated with average lethal concentrations of NaCl and sorbite. Also, the irradiated seeds were treated with half the LD_{50} concentration of each of the stress factors together.

To see the effect of the abiotic stress and irradiation control plants were treated as plants from processed seeds.

3. Results

As a result of the research, it was discovered that the median lethal dose (LD50) of γ-rays for rice is 100 Gy, and for fast neutrons – 50 Gy. The median lethal concentration of NaCl is 1.0% aqueous solution, and for sorbite – 7.5% [22].

In terms of seed germination, the largest numbers of germinating grains in all varieties were obtained from NaCl variants when, 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 sorbite treatment (Figure 1).

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

However, 10 days after the seedlings' emergence, despite, the of seedling's watering termination with NaCl and sorbite solutions, most seedlings began to die, especially gamma-ray irradiated species. As a result, 20 days after the 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 before to transplantation most distinguishable results were as follows: the Aikerim variety with NaCl (3 and 6 pcs.), the Leader variety with sorbite (6-12 pcs.) and NaCl + sorbite (8 and 14 pcs.), the Syr Suluy variety has variants with NaCl (9 - 30 pcs.) and NaCl + sorbite (12 and 30 pcs.)

Plants that survived gamma rays and fast neutrons treatment, as well as, salinization (NaCl) and drought (sorbite) factors and placed in the special paddy-field containers during their tillering phase, were further transplanted directly into the soil. During the 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 photographs.

The results of the phenological observations revealed that almost all altered forms had interphase periods of prolongation and retardation in the growth and development of the plants, except for the individual mutant plants of the cultivar Aikerim (Table 1). The observed very high level of panicle emptiness and low plant growth confirm the manifestation of mutational processes under the influence of ionizing radiation.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 19 April 2023 doi:10.20944/preprints202304.0590.v1

Table 1. 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 (sorbite), 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
		sorbite	13	33	32	43	121
		NaCl+ sorbite	13	33	32	44	122
	Fast neutrons	NaCl	12-13	32-34	33-35	43-46	120-128
		sorbite	13	32	35-36	44	124-125
		NaCl+ sorbite	12-13	31-32	36-37	43-45	122-127
	Control	-	12	32	30	42	116
Syr Suluy	γ – rays	NaCl	No shoots				
		sorbite	12-13	33-35	31-33	44-46	120-127
		NaCl+ sorbite	12-13	32-35	31-33	44-45	119-126
	Fast neutrons	NaCl	12	34	33	45	124
		sorbite	12-13	32-33	31-33	44-16	119-125
		NaCl+ sorbite	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 the vegetation period for the Aikerim variety by 3-12 days, for the Leader variety by 1-9 days and 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 2).

Table 2. – 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.**		Empti-ness,%	Grain weight, g	
					full	**puny**			
1	**2**	**3**	**4**	**5**	**6**	**7**	8	9	10
Aikerim variety									
Control	121	4,4	4,4	19,6	107	42	28,2	3,17	11,79
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									
Control	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									
Control	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	16,6-100,0	0,00-1,29	0,00-3,39
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 – sorbite; 3 – NaCl + sorbite. .

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.) varieties. 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 11; 30; 74 pcs, respectively., in total – 115 pcs.

Of these, those resistant to NaCl are: from the Aikerim variety – 10 pcs., Leader variety – 1 pcs. and Syr Suluy – 30 pcs. Resistant to sorbite: Aikerim variety – 3 pcs., in the Leader variety – 19 pcs. and the variety Syr Suluy – 17 pcs. Resistant to NaCl + sorbite: in the variety Aikerim – 3 pcs., in the variety Leader – 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, an increase of up to 127 cm and a decrease of up to 98 cm. Whereas the Leader and Syr Suluy varieties, mutants were 11-41 cm lower than the originals (Fig. 2).

Aikerim variety | Leader variety | Syr Suluy variety

Figure 2. Photo of the control plants (on the left) and M1 plants distinguished by resistance to salinity and drought.

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 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.

4. Discussion

It has been established during the irradiation of rice varieties like Aikerim, Leader 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 was NaCl – 1,0 %; sorbite – 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 1 to 30 plants that were transplanted into especially 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 Aikerim (16 pcs.). The number of mutants received from exposure of gamma rays was 10 pcs in the Aikerim 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. 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.

Funding: This research is funded by the Ministry of Agriculture of the Republic of Kazakhstan (No. BR10765056).

References

1. Issanova, G. T.; Abuduwaili, J.; Mamutov, Zh. U.; Kaldybaev, A. A.; Saparov, G. A.; Bazarbaeva, T. A. Saline soils and identification of salt accumulation provinces in Kazakhstan, *Arid Ecosyst*. **2017**, 7, 4, pp. 243–250.
2. Karlykhanov, O. K.; Toktaganova, G. B. The Assessment of Irrigated Land Salinization in the Aral Sea Region, *Sci. Educ*. **2016**.
3. Suska-Malawska, M. et al. Spatial and In-Depth Distribution of Soil Salinity and Heavy Metals (Pb, Zn, Cd, Ni, Cu) in Arable Irrigated Soils in Southern Kazakhstan, *Agronomy* **2022**, 12, 5, p. 1207.
4. 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.
5. Abugalieva, S.; Baibosynova, S.M.; Kondybaev, A.B.; Podolskih, A.N.; Turuspekov, Y.; Genetic and phenotypic diversity of the rice collection in Kazakhstan. **2014**., *175*, pp. 46–59
6. Abikenova, S. M.; Rau, A. G.; Assanbekov, B. A.; Zhanashev, I. Z.; Kalybekova, E. M. Research of the rice productivity on saline lands of rice systems in Kazakhstan Republic, *Life Sci. J.* **2014**, 11, pp. 356–361.
7. Olzhabayeva, A. O.; Rau, A. G.; Sarkynov, E. S.; Baimanov, Z. N.; Shomantaev, A. A. Effect of Irrigation and Fertilizers on Rice Yield in Conditions of Kyzylorda Irrigation Array, *Biosci. Biotechnol. Res. Asia* **2016**, 13, 4, pp. 2045–2053.
8. Haque, M.A.; Rafii, M.Y.; Yusoff, M.M.; Ali, N.S.; Yusuff, O.; Datta, D.R.; Anisuzzaman, M.; Ikbal, M.F. Advanced Breeding Strategies and Future Perspectives of Salinity Tolerance in Rice. *Agronomy* **2021**, 11, 1631. doi: 10.3390/agronomy11081631.
9. Sikora, P.; Chawade, A.; Larsson, M.; Olsson, J.; Olsson, O. Mutagenesis as a Tool in Plant Genetics, Functional Genomics, and Breeding, *International Journal of Plant Genomics* **2011**, 2011, pp. 1–13, doi: 10.1155/2011/314829.
10. Oladosu, Y.; Rafii, M. Y.; Abdullah, N.; Hussin, G.; Ramli, A.; Rahim, H. A.; G. Miah & Magaji Usman. Principle and application of plant mutagenesis in crop improvement: a review, *Biotechnology & Biotechnological Equipment* **2016**, 30:1, pp. 1-16. doi: 10.1080/13102818.2015.1087333.

11. Mutant Variety Database FAO/IAEA-MVD. Food and agriculture organization of the United Nations/ International atomic energy agency – mutant variety database. Available online: https://nucleus.iaea.org/sites/mvd (accessed on 5 April 2023).
12. Bakirov, K.; Vereshchagin, G.A. The use of mutagenic factors in the creation of new varieties of rice. *Bulletin of Agricultural Sciences of Kazakhstan* **1980**, 12, pp. 20-26.
13. 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* **2016**, 1(65).
14. Kharitonov, E.M.; Goncharova, Y.K.; Maliuchenko, E.A. The genetics of the traits determining adaptability to abiotic stress in rice (Oryza sativa L.). *Russ J Genet Appl Res* **2017,** 7, pp. 684–697. doi: 10.1134/S2079059717060089
15. 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, Kazakhstan, 2019.
16. Su, Y.; Zhao, G.; Wei, Z.; Yan, C.; Liu, S. Mutation of cellulose synthase gene improves the nutritive value of rice straw. *Asian-Australasian journal of animal sciences* **2012**, 25(6), pp. 800–805. doi: 10.5713/ajas.2011.11409.
17. Viana, V.E.; Pegoraro, C.; Busanello, C.; Costa de Oliveira, A. Mutagenesis in Rice: The Basis for Breeding a New Super Plant. *Front. Plant Sci.* **2019**, 10, 1326. doi: 10.3389/fpls.2019.01326.
18. Cheng, Z.; Lin, J.; Lin, T.; Xu, M.; Huang, Z.; Yang, Z.; Huang, X.; Zheng, J. Genome-wide analysis of radiation-induced mutations in rice (Oryza sativa L. ssp. indica). *Mol. Biosyst.* **2014**, 10, pp. 795–805. doi: 10.1039/C3MB70349E.
19. Schiocchet, M. A.; Noldin, J. A.; Raimondi J. V.; Neto, A. T.; Marschalek, R.; Wickert, E.; Martins, G. N. et. al. SCS118 Marques — New rice cultivar obtained through induced mutation. *Crop Breeding and Applied Biotechnology* **2014**, 14, pp. 68-70. doi: 10.1590/S1984-70332014000100012.
20. Ichida, H.; Morita, R.; Shirakawa, Y.; Hayashi, Y.; T. Abe. Targeted exome sequencing of unselected heavy-ion beam-irradiated populations reveals less-biased mutation characteristics in the rice genome. *The Plant Journal* **2019**, 98, 2, pp. 301-314. doi: 10.1111/tpj.14213.
21. Li, F.; Shimizu, A.; Nishio, T.; Tsutsumi, N.;. Kato, H. Comparison and Characterization of Mutations Induced by Gamma-Ray and Carbon-Ion Irradiation in Rice (Oryza sativa L.) Using Whole-Genome Resequencing, *G3 (Bethesda, Md.)* **2019**, 9, 11, pp. 3743–3751. doi: 10.1534/g3.119.400555.
22. Abdelnour-Esquivel, A.; Perez, J.; Rojas, M. et al. Use of gamma radiation to induce mutations in rice (Oryza sativa L.) and the selection of lines with tolerance to salinity and drought. *In Vitro Cell.Dev.Biol.-Plant* **2020**, 56, pp. 88–97. doi: 10.1007/s11627-019-10015-5.
23. Mei, L. Effect of Fast Neutron Irradiation on Amylose Content and Amylopectin Structure of Thailand Rice Cultivar Jao Hom Nin. *Agricultural Science&Technology* **2010**, 4, pp. 19-21.
24. Ruengphayak, S.; Ruanjaichon, V.; Saensuk, C. et al. Forward screening for seedling tolerance to Fe toxicity reveals a polymorphic mutation in ferric chelate reductase in rice. *Rice* **2015**, 8. doi: 10.1186/s12284-014-0036-z.
25. Li, G. *et al.*, 'Genome-Wide Sequencing of 41 Rice (Oryza sativa L.) Mutated Lines Reveals Diverse Mutations Induced by Fast-Neutron Irradiation', *Mol. Plant* **2016**, 9, 7, pp. 1078–1081. doi: 10.1016/j.molp.2016.03.009.
26. Li, G. *et al.*, 'The Sequences of 1504 Mutants in the Model Rice Variety Kitaake Facilitate Rapid Functional Genomic Studies', *Plant Cell* **2017**, 29, 6, pp. 1218–1231. doi: 10.1105/tpc.17.00154.
27. 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", Obninsk, Russia, 18-21 October 2022.
28. Bakiruly, K.; Tautenov, I.A.; Zhalbyrov, A.E. Creation of source material for rice breeding by treating seeds with ionizing radiation. *Bulletin of the Korkyt Ata Kyzylorda University* **2022**, 3(62), pp. 55-64.
29. Kruglyak, A.I.; Doroshkevich, A.S.; Aleksiayenak, Yu.; Appazov, N.O.; Bakiruly, K.B.; Balasoui, M.; Mirzayev, M.N.; Nabiyev, A.A.; Popov, E. 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, Russia, 25-29 April 2022.

 doi:10.20944/preprints202304.0590.v1

30. Doroshkevich, A.S.; Zelenyak T.Yu.; Kruglyak, A.I.; Alekseenok, Yu.V. et. al. 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: The 16th International Symposium on Origin of Matter and Evolution of Galaxies, Hanoi, Vietnam, 25-28 October 2022.
31. Kruglyak, A.I.; Doroshkevich, A.S.; Zelenyak T.Yu.; Alekseenok, Yu.V. et. al. Influence of cosmogenic neutron radiation on the evolution of terrestrial biological forms on the example of rice and Oyster mushrooms cultures // Book of Abstract: The 16th International Symposium on Origin of Matter and Evolution of Galaxies, Hanoi, Vietnam, 25-28 October 2022.
32. Lyahovkin, A.G. Methodological guidelines for the study of the world collection of rice and the classifier of the genus Oryza L, Vsesoyuz. akad. s.-h. nauk im. V. I. Lenina: Vsesoyuz. nauch.-issled. in-t rastenievodstva im. N. I. Vavilova, Leningrad, USSR, 1974; p. 25.
33. Dospekhov, B.A. Methodology of field experience, , 5rd ed.; Agropromizdat: Moscow, USSR, 1985; p. 351